

# THE IMMUNOLOGY OF MUCOSAL MODELS OF INFLAMMATION<sup>1</sup>

---

Warren Strober,<sup>1</sup> Ivan J. Fuss,<sup>1</sup> and Richard S. Blumberg<sup>2</sup>

<sup>1</sup>*Mucosal Immunity Section, Laboratory of Clinical Investigation, NIAID, NIH, Bethesda, Maryland 20892-1890; e-mail: wstrober@niaid.nih.gov*

<sup>2</sup>*Division of Gastroenterology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115*

**Key Words** Crohn's disease, ulcerative colitis, tolerance, cytokines, Th1/Th2

■ **Abstract** In recent years the status of the inflammatory bowel diseases (IBDs) as canonical autoimmune diseases has risen steadily with the recognition that these diseases are, at their crux, abnormalities in mucosal responses to normally harmless antigens in the mucosal microflora and therefore responses to antigens that by their proximity and persistence are equivalent to self-antigens. This new paradigm is in no small measure traceable to the advent of multiple models of mucosal inflammation whose very existence is indicative of the fact that many types of immune imbalance can lead to loss of tolerance for mucosal antigens and thus inflammation centered in the gastrointestinal tract. We analyze the immunology of the IBDs through the lens of the murine models, first by drawing attention to their common features and then by considering individual models at a level of detail necessary to reveal their individual capacities to provide insight into IBD pathogenesis. What emerges is that murine models of mucosal inflammation have given us a road map that allows us to begin to define the immunology of the IBDs in all its complexity and to find unexpected ways to treat these diseases.

## INTRODUCTION

The study of animal models of mucosal inflammation as a means to probe the pathogenesis of inflammatory bowel disease (IBD) extends back almost a half century [for reviews of the older literature see Strober (1) and Kim & Berstad (2)], and it is fair to say that this kind of study embodied the first serious attempt to determine the immunologic basis of this category of disease. One class of early models is that devised by Kirsner and his colleagues in the early 1960s in which the mucosal immune system was manipulated in some way to cause a

---

<sup>1</sup>The US Government has the right to retain a nonexclusive, royalty-free license in and to any copyright covering this paper.

mucosal (colonic) inflammation (3). Perhaps the most interesting of these models consisted of colonic inflammation that was induced in rabbits via the “Auer procedure,” wherein rabbits are first immunized with an antigen (such as OVA), then subjected to disruption of the colonic epithelial barrier with formalin, and finally are re-administered the original antigen by a local mucosal or system route (4). This procedure led to a colonic inflammation not unlike that in ulcerative colitis but was transient even when the procedure was repeated in the same animal. A more sustained inflammation, however, was obtained in the late 1970s by Mee et al., who modified the Auer procedure by sensitizing animals (rabbits) to an *Escherichia coli*-associated antigen (5). Similarly, in studies performed some 10 years earlier, now almost forgotten, Halpern et al. showed that immunization of rats with live or dead *E. coli* (in Freund’s adjuvant) led to chronic colitis even without introduction of a colonic irritant per rectum; in addition, feeding of *E. coli* prevented the development of colitis (6). These studies, together with early studies of dinitrochlorobenzene-induced colitis reported about the same time as the studies of Mee et al. and coworkers (5, 7, 8), clearly indicated that an initial immunologic assault of varying cause on the gastrointestinal tract can lead to more sustained inflammation as a result of a break in normal “tolerance” to antigens in the mucosal microflora.

Another class of early models of mucosal inflammation were those produced by physical agents and included colitides produced by exposure to acetic acid, phorbol ester, F-met-leu-phe, and various sulfated polysaccharides such as carageenan, amylopectin sulfate, and dextran sulfate sodium (DSS) (9–20). One common feature of these agents appears to be their capacity to disrupt the epithelial cell barrier and therefore to promote increased cellular exposure to normal mucosal microflora. Evidence for this comes from studies of DSS-induced colitis in which it has been shown that DSS alters mucosal barrier function prior to the onset of colitis (19). In addition, colitis caused by exposure to F-met-leu-phe has also been shown to be associated with changes in barrier function, in this case mediated by neutrophils (14). One possible or even probable consequence of this change in barrier function is that mucosal phagocytes become subject to activation by substances in the mucosal flora and this, in turn, leads to antigen nonspecific release of pro-inflammatory cytokines (e.g., TNF- $\alpha$ ) and inflammation. This scenario is supported by the observation that both DSS colitis and carageenan colitis can be effectively treated with antibiotics (20, 21).

Disruption of barrier function(s) as a mechanism in physical agent-induced colitis fits with a second common feature of colitides caused by physical agents, namely their relative independence from lymphocyte-mediated responses. Thus, in DSS-induced colitis, it is evident that mice lacking T cells, B cells, and NK cells can still develop colitis in response to DSS (22). This being said, in the presence of an intact immune system containing these cellular elements, dextran sulfate leads to activation of lymphocytes and the induction of Th1 and/or Th2 responses. This leads to the conclusion that in physical agent-induced colitis a T cell-mediated inflammation can be superimposed on macrophage-induced inflammation.

This brief survey of historically important models of mucosal inflammation leaves little doubt that on close reading they provide data that presaged many of the findings obtained with a set of newer models that had been extensively characterized over the past decade. Thus, as alluded to above, these models revealed the critical role of the mucosal microflora in the pathogenesis of mucosal inflammation and the related role of barrier function as a bulwark against extensive stimulation of the mucosal immune system by the microflora. In addition, they provide the first insights into the often overlooked role of antigen nonspecific factors in mucosal inflammation and even provide an early hint of the role of active tolerance in preventing such inflammation [the feeding studies of Halpern et al. (6)]. These considerations, of course, are by no means meant to belittle the new knowledge of mucosal inflammation (and by extension IBD) that have come from studies of newer models. Not only have the latter provided for the first time a detailed framework for the understanding of the various proinflammatory and antiinflammatory mechanisms at work in this type of inflammation, but they have also provided us with invaluable clues as to how the latter can be effectively treated in humans.

In discussing these newer models of mucosal inflammation, we first survey their common features to derive basic principles of mucosal inflammation that are applicable to this area of study as a whole. We then discuss major individual models in depth, emphasizing the particular insights derivable from each model and how each model helps establish the basic principles and mechanisms of mucosal inflammation. This, the main body of the review, is subdivided into sections on Th1 models, Th2 models, and barrier function models.

## BASIC (GENERAL) FEATURES OF MODELS OF MUCOSAL INFLAMMATION

As is evident from the detailed review of individual models of mucosal inflammation that follows, certain recurrent principles emerge that relate to all models to a greater or lesser extent. These principles together define the basic immunology of both models of mucosal inflammation and of human IBD, and thus it is useful to discuss them first in an outline form that can later be fleshed out in the discussions of individual models to follow.

### Final Common Pathways of Mucosal Inflammation

As becomes amply evident below, models of mucosal inflammation reflect a remarkably wide variety of causes. Nevertheless, the resulting inflammation that develops is almost always channeled into a final common pathway of inflammation, mediated by either an excessive Th1 T-cell response associated with excessive IL-12/IFN- $\gamma$ /TNF- $\alpha$  secretion or an excessive Th2 T-cell response associated with increased IL-4/IL-5 secretion (reviewed in 23). The great majority of models are in fact Th1 models, but why this is the case is far from clear (see Table 1). One factor may relate to the influence of strain on disease because the given model may

**TABLE 1** Models of mucosal inflammation classified by nature of T cell-mediated inflammation

Th1 Models	Th2 Models
TNBS colitis (SJL/J mice)	TCR- $\alpha$ chain deficiency
SCID-transfer colitis	TNBS colitis in BALB/c mice**
TCR Tg mice with lymphopenia	Oxazalone colitis
IL-10 deficiency colitis <sup>a</sup>	WASP deficiency
IL-10 signaling defects (CRF2-4 deficiency)	
Tg <sub>c</sub> 26 mice	
TNF <sup><math>\Delta</math>ARE</sup> mice (TNF- $\alpha$ overproduction)	
C <sub>3</sub> H/HeJBir mice	
Gi2 $\alpha$ -deficient mice	
Samp1/Yit mice	
T-bet Tg mice	
STAT4 Tg mice	
TGF- $\beta$ RII dominant-negative Tg mice	
HLA-B27 Tg rats	
<i>Mdr1a</i> -deficient mice	
DSS colitis	
IL-7 Tg mice	

<sup>a</sup>Abbreviations: SCID, severe combined immunodeficiency; TCR, T cell receptor; CRF2-4, cyto receptor family 2-4; TNF, tumor necrosis factor; STAT-4, signal transduction and activators of transcription-4; TGF, transforming growth factor; DSS, dextran sulfate sodium; WASP, Wiskott-Aldrich syndrome protein.

\*\*Mixed response but initially Th1, later Th2.

Abbreviations: TNBS, trinitrobenzene sulfonic acid; SCID, xx; TCR, T cell receptor; CRF2-4, xx; TNF, xx; STAT4, xx; TGF, xx; HLA-B27, xx; DSS, xx; WASP, xx.

manifest a Th1 character in the SJL/J strain mouse but may manifest a Th2 character (or mixed Th1/Th2 character) in the BALB/c mice. A more likely explanation, however, relates to the fact that in most if not all models the inflammation is driven by antigens in the normal mucosal microflora, which in effect means that it will be influenced by mitogens [e.g., lipopolysaccharides (LPS), CpGs] and superantigens associated with these organisms that tend to induce IL-12 production and thus Th1 responses. This is nicely illustrated by trinitrobenzene sulfonic acid (TNBS)-colitis in SJL/J mice that manifest increased LPS-driven IL-12 responses, which are thought to play a key role in the Th1 response elicited by TNBS administration (see discussion below; G. Bouma & W. Strober, unpublished observations).

A final possibility is that many of the models are due to a failure to regulate mucosal responses that are essentially normal responses to antigens in the mucosal milieu. As we see below, such regulation most likely involves the secretion of

suppressor cytokines such as TGF- $\beta$  (or IL-10), which is more closely related to the regulation of Th1 responses than Th2 responses (24, 25). In fact, there is considerable evidence that Th1 responses suppress both the expansion of TGF- $\beta$ -secreting cells and TGF- $\beta$  signaling (26, 27) and, contrariwise, TGF- $\beta$  interferes with IL-12 signaling (28–30). Thus, Th1 and TGF- $\beta$  responses have a reciprocal relationship to one another and appear to be mutually exclusive. On the other hand, there is some evidence that Th2 and TGF- $\beta$  responses can co-exist and that it requires higher levels of TGF- $\beta$  to suppress a Th2 response than a Th1 response (25, 31). These considerations suggest that Th1-mediated mucosal inflammations are more sensitive to defects of regulation (mediated by TGF- $\beta$ ) and thus, defects in regulation will more frequently lead to Th1-mediated inflammation than Th2-mediated inflammation.

The bias of the experimental models toward Th1-mediated inflammation raises the question as to when and how Th2-mediated inflammation ever occurs. One factor is again the nature of the antigen driving the inflammation or, alternatively, the specificity of the T cell receptor (TCR) on the reactive T cells. In this regard, certain antigens are “Th2-type antigens,” perhaps because the nature of the antigen dictates the type of antigen presenting cell that induces T cell differentiation in Th2 T cells. Evidence for this is inherent in the fact that one haptenating agent, TNBS, elicits a Th1 response in SJL/J mice, whereas another, oxazalone, elicits a Th2 response (31, 32). In addition, a Th2-oriented response may result in colitis associated with TCR- $\alpha$  chain deficiency because in this situation the T cells utilize a TCR (a  $\beta\beta$  TCR) that may have the ability to recognize and expand in response to antigens only under conditions that allow Th2 responses (33, 34). One thing to keep in mind, however, is that the Th1-mediated inflammation may switch to a Th2 inflammation under some circumstances. This is seen in IL-10-deficient mice, perhaps because in the absence of IL-10, cells in which IL-4 signaling leads to GATA-3 suppression of IL-12 signaling gradually accumulate, and ultimately a Th2 T cell dominates the inflammation (35; A.D. Levine, personal communication).

Whether a Th1 or a Th2 response is responsible for the mucosal inflammation has considerable impact on the nature of the inflammation because, as we see below, Th1 responses are marked by transmural cellular infiltration that in some cases is associated with granulomata (i.e., TNF $^{\Delta ARE}$  model and SAMP1/Yit model) (36–38), and whereas epithelial cell layer changes are clearly present, they are not a dominant feature. A similar histopathologic picture is obtained in Crohn’s disease, and thus it is fair to say that, in general, Th1 models are related to this human disease (23). This presumed association between Th1 models and Crohn’s disease is also strengthened by the fact that Crohn’s disease is in fact a Th1-mediated inflammation (39–42). Th2-mediated inflammations are, on the contrary, marked by more superficial cellular infiltrates associated with a greater disruption of the epithelial layer and in some cases greater polymorphonuclear infiltration. This situation is more akin to ulcerative colitis, but this correlation is inexact because ulcerative colitis has not been clearly shown to be a Th2-mediated inflammation. Thus, whereas some authors have found high IL-5 levels in ulcerative colitis, IL-4

levels are quite normal. Clearly, if a Th2 inflammation is present in ulcerative colitis, it is a highly atypical Th2 inflammation (39, 40).

## Cellular Elements Involved in Mucosal Inflammation

**ANTIGEN PRESENTING CELLS** Antigen presenting cells (APCs) in mucosal tissues are probably key cells in the induction of both mucosal effector and regulatory cell responses. Hence, it is likely (but not yet proven) that defects of T cell responses arise either from defects in APC function or APC–T cell interactions. Alternatively, it is possible that APCs are the target of regulatory cells, a possibility proposed by Malmstrom et al. in relation to OX40-positive APCs present in the mesenteric nodes of mice with SCID-transfer colitis (43).

Macrophages, a type of APC, are activated in mucosal inflammation and function mainly as effector cells. However, these cells may also be involved in regulatory interactions. This possibility is realized in mice with myeloid cell-specific STAT3 deficiency that have macrophages that cannot produce several STAT3-dependent cytokines, such as the important regulatory cytokine, IL-10 (44). Thus, *in vitro* macrophages in this model of inflammation exhibit heightened effector activity characterized by increased LPS-induced production of IL-12, TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ; thus, *in vivo* these macrophages lead to LPS-induced mucosal inflammation.

**T CELLS** T cells play multiple roles in experimental mucosal inflammation both as effector cells and regulatory cells. The former are mainly CD4+ T cells because these cells make up the main cell populations that infiltrate mucosal tissues in all models so far studied and because in instances in which they are deleted *in vivo*, inflammation is ameliorated (45). CD8+ T cells are also present in tissues but do not appear to play a decisive pathologic role because in the few instances in which they were deleted *in vivo* no major effect on inflammation was obtained (46). This does not, however, rule out a supportive pathogenic role because increased cytotoxic T cell function has been observed in some of the models (47). Evidence has recently appeared that indicates that loss of epithelial cells in ulcerative colitis can be attributed to a T cell or NK cell-mediated cytotoxic event (48). The above information on the role of cytotoxicity in models would suggest, however, that even if cytotoxic elimination of epithelial cells occurs in ulcerative colitis, such cytotoxicity is not likely to be a major component of the overall immunopathologic process.

$\gamma\delta$  T cells, *i.e.*, T cells confined to the intra-epithelial compartment, do not play an important role as effector cells in any form of colitis, except perhaps in TCR- $\alpha$  chain-deficient mice, in which they are present in increased numbers (49). In recent studies it has been shown that whereas  $\gamma\delta$  T cells cannot in themselves induce colitis (in lymphopenic TCR- $\alpha$  chain-deficient mice), injection of anti-TCR $\delta$  antibody into TCR- $\alpha$  chain-deficient mice prevented development of colitis (50). On this basis,  $\gamma\delta$  T cells in this context appear to play an accessory

role in the inflammation.  $\gamma\delta$  T cells also do not have a major regulatory role in mucosal inflammation, as  $\gamma$  chain-deficient mice do not develop inflammation (51). Here too, however, one can find an exception, in that mice lacking  $\gamma\delta$  T cells are reported to develop more severe TNBS-colitis (52). Finally, one very definite and in this case positive role of  $\gamma\delta$  T cells in mucosal inflammation is their role in the healing of mucosal inflammation. This is shown by the fact that intra-epithelial  $\gamma\delta$  T cells produce factors, notably keratinocyte growth factor, that may facilitate restoration of epithelial cell barrier integrity in DSS-colitis (53).

Whereas, as indicated above, CD4+ T cells can function in the various models as either Th1 or Th2 effector cells, they can also function as regulatory cells. With regard to the latter, several different types of cells have been described, but it is very possible that these are in reality one cell that appears in different disguises. One type of regulatory cell is a TGF- $\beta$ -secreting T cell (a so-called Th3 cell), which is the cell induced by antigen feeding during the development of oral tolerance. The mucosal cytokine milieu necessary for the induction of this cell is not well understood, although it is known that Th2 conditions favor induction and Th1 conditions inhibit induction (reviewed in 24, 26, 27). IL-10 has been seriously considered as a possible inductive cytokine for this cell, but in recent studies of oral tolerance induction as well as in *in vitro* studies of Th3 T cell development from naive cells, IL-10 has no direct inductive effect on the development of Th3 T cells and may enhance TGF- $\beta$  production only through its capacity to down-regulate Th1 responses. However, in the same *in vitro* studies TGF- $\beta$  itself had a positive autocrine effect on its own secretion (54). A second type of regulatory cell is an IL-10 secreting cell (a Tr1 cell), which may also secrete small amounts of TGF- $\beta$  (55). This cell has poor proliferative capacities and in initial studies was induced by sequential antigenic restimulation in the presence of IL-10. More recently, however, it has been shown that both IL-10 and IFN- $\alpha$  are necessary for its induction (56).

Yet another regulatory cell is the CD25+ T cell, which is a thymus-derived cell that inhibits effector T cells via cell-cell contact rather than secretion of an inhibitory cytokine (57, 58). Recently, however, Nakamura et al. have reported data that show that most CD25+ T cells bear surface TGF- $\beta$  in the form of a latent TGF- $\beta$  protein (TGF- $\beta$  associated with latency-associated protein) and secrete TGF- $\beta$  and IL-10 when activated in the presence of IL-2 and/or strong costimulatory signals (59). These authors suggest that under minimal stimulation conditions that might occur prior to inflammation, these cells inhibit via cellular contact and activation of the surface TGF- $\beta$  at the cell-cell interface. In contrast, they suggest that under maximal stimulation conditions that occur in the presence of inflammation, CD25+ T cells inhibit via secretion of TGF- $\beta$  and IL-10. Thus, the CD25+ T cells have qualities of both Th3 and Tr1 regulatory cells.

A final type of suppressor cell is the NK cell or NK-T cell. The former has been shown to suppress inflammation in the SCID-transfer model of colitis (60), whereas the latter has been shown to suppress inflammation in DSS colitis (61). The NK-T cell preferentially recognizes glycolipid antigens presented via an atypical

MHC class I molecule (CD1d), which is ordinarily expressed on dendritic cells, B cells, and epithelial cells; thus, this cell may be activated via antigens presented by both epithelial cells and more conventional APCs (62). The mechanism by which NK or NK-T cells suppress mucosal inflammation or other forms of inflammation is poorly understood, as their possible cellular targets of suppression are presently unknown.

**B CELLS** Whereas autoantibodies are found in some models of colitis as well as in human IBD, it does not appear that B cells play a role either in induction of mucosal inflammation or its maintenance. In fact, in the one instance that B cells have been actively studied, in the Th2-mediated inflammation in TCR- $\alpha$  chain deficiency, they appear to play a protective role rather than a pathologic role (see below) (63). Whether such protection also occurs in human IBD is not known.

**EPITHELIAL CELLS** Epithelial cells form a barrier against exposure to mucosal microflora and other mucosal antigens and thus play a key role in the down-regulation of mucosal immune response. As is evident from the discussion of individual models of mucosal inflammation below, in several models alterations in this barrier are the primary cause of colitis (64, 65), whereas in several other models a change in barrier function is a contributory (secondary) factor (66). Epithelial cells also function as sensors of the bacterial microenvironment and release chemokines in a programmed fashion when in contact with pathogens. Such chemokine release has the effect of drawing leukocytes into peri-epithelial sites, which then set up the first line of defense against invading organisms. Whether such chemokine release also plays a role in the initiation of mucosal inflammation is not yet clear. That it may is suggested by a recent study of *Mdr1a*-deficient mice, i.e., mice whose epithelial cell cannot expel proteins from within the cell including those proteins derived from infectious pathogens (65). As described below, such mice develop colitis that is likely to be due to prolonged secretion of chemokines and cytokines rather than a break in the epithelial cell barrier per se.

## Broad Categories of Mucosal Models of Inflammation

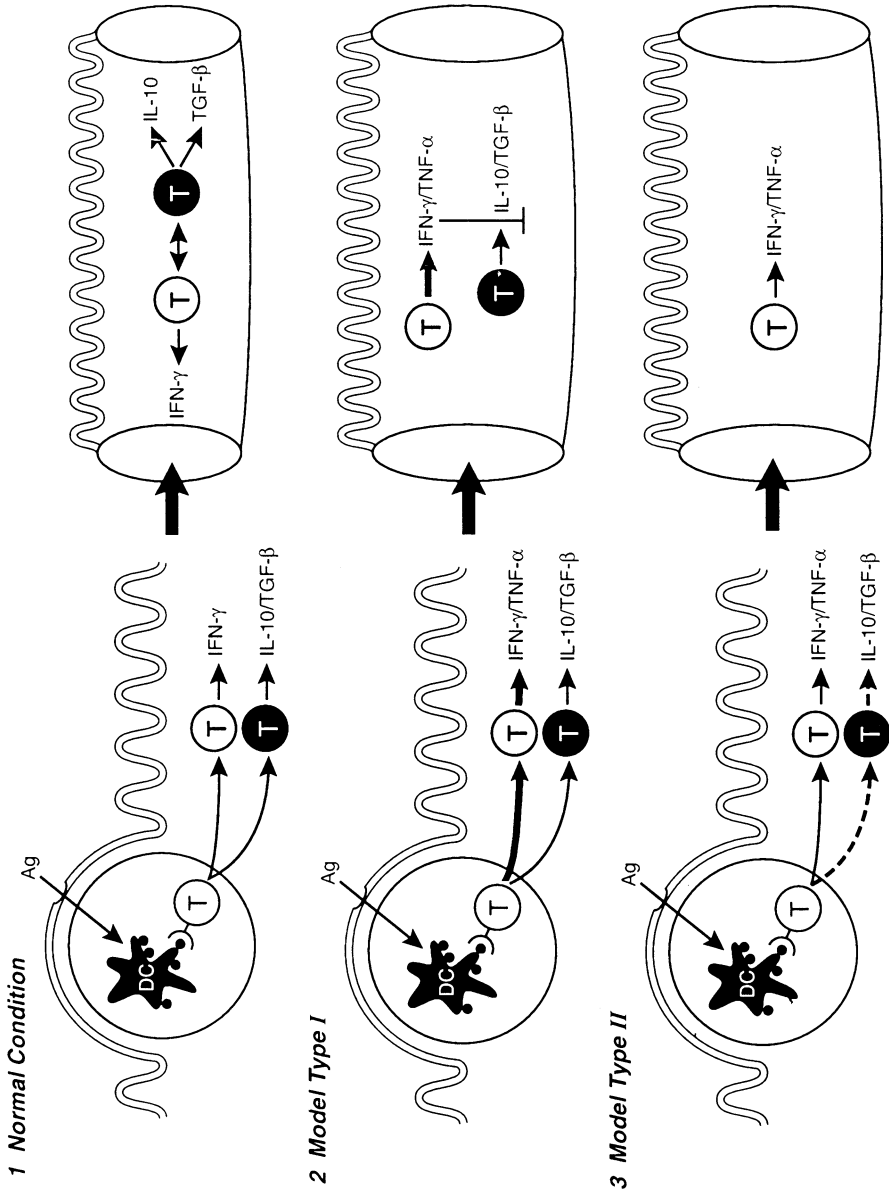
As discussed in several previous reviews (24, 67–69), mucosal immune responses are fine-tuned by opposing immune mechanisms that on the one hand lead to effector cell responses addressing host defense at mucosal surfaces and on the other to tolerogenic responses preventing inflammatory reactions to the myriad of antigens in the mucosal environment. It is now apparent that the tolerogenic response has two major components: (a) processes by which mucosal antigens (in the form of unadjuvanted proteins) bring about “classical” tolerance via induction of T cell anergy or deletion either in the mucosal tissues per se or upon gaining entrance to the circulation and the central lymphoid tissues; and (b) processes by which mucosal antigen induces regulatory T cells, which secrete antigen-nonspecific suppressor

cytokines such as IL-10 and TGF- $\beta$ . These two tolerogenic processes operate in tandem and are probably both necessary to maintain mucosal homeostasis. Thus, whereas induction of anergy/deletion can greatly reduce the number of T cells that can respond to a mucosal antigen, it is probably not able to eliminate all such T cells, and the latter become memory cells potentially able to evoke inflammatory responses. These latter cells are held in check, however, by a cadre of regulatory T cells that respond to the same stimulating antigen.

We must bear in mind that the main antigenic pool to which mucosal homeostasis must apply is the antigenic pool associated with the mucosal microflora, which by their persistence and proximity are formally equivalent to self antigens. Given the inevitable inefficiency of a deletion mechanism in relation to so large and mutable an antigenic pool and given the fact discussed above that a full scale effector cell response tends to obliterate a regulatory response (at least for a period of time), the burden (or the challenge) of the mucosal tolerogenic mechanism may fall disproportionately on regulatory cell function. This view is amply supported in the discussions of the individual models below in which the origin of the inflammation can be repeatedly traced to an inadequate regulatory response rather than to a hyperactive or excessive effector response to antigens in the mucosal microflora.

The mechanisms governing the development of mucosal tolerance (also called oral tolerance) are not yet completely understood. One important mechanism probably relates to the special nature of the mucosal dendritic cell population, which may have an increased propensity to present antigen in a way that induces either anergic/deletional tolerance or suppressor cell tolerance. Recent work showing that subsets of mucosal dendritic cells have a substantially different cytokine secretion profiles than spleen dendritic cells, i.e., produce more IL-10, supports this concept (70, 71). Nevertheless, much remains to be learned about the origin of these cells and how they shape mucosal responses. In particular, it is not known how these cells are influenced in their development by the adjacent epithelium and how, in turn, these cells induce either the de novo development of regulatory cells or the expansion of a preexisting population of regulatory cells.

In any case, the above considerations allow us to classify models of mucosal inflammation into two broad categories (Figure 1): "type 1 models," wherein the defect lies with the effector mechanisms of the mucosal response and "type 2 models," wherein the effector cell response is normal, but the regulatory cell response is impaired. One example of a type 1 model is the colitis seen in mice bearing a STAT4 transgene (72). These mice have an increased propensity to mount a Th1 T cell response because of excessive responsiveness to IL-12 signaling; thus, when T cells from these mice are exposed to autologous bacterial antigen *in vitro* and then transferred to a SCID recipient, they induce colitis in the recipient, whereas naive T cells from normal mice do not. A second example is TNBS colitis in SJL/J mice, wherein it is thought that the colitis is preceded by and is dependent on a genetically determined IL-12 hyperresponsiveness ignited by a disturbance of epithelial barrier function by ethanol followed by exposure of the mucosal APCs



**TABLE 2** Models of mucosal inflammation<sup>a</sup>

Type I models	Type II models
TNF <sup>ARE</sup> mice	SCID-transfer colitis
TNBS colitis	IL-10 deficiency and IL-10 signaling defect colitis
?C <sub>3</sub> H/HeJBir mice	?C <sub>3</sub> H/HeJBir mice
Gi2 $\alpha$ -deficient mice	IL-2-deficient mice
STAT4 Tg mice	TGF- $\beta$ RII dominant-negative mice
N-cadherin dominant-negative mice	Tg <sub>e</sub> 26 mice
IL-7 Tg mice	
DSS colitis	
Mice with NF- $\kappa$ B defects	

<sup>a</sup>Unidentified: SAMPI/Yit mice; HLA-B27 Tg rats, mice with Wiskott-Aldrich syndrome protein deficiency.

Abbreviations: TNF, xx; TNBS, trinitrobenzene sulfonic acid; STAT4, xx; DSS, dextran sulfate sodium; SCID, xx; TGF, xx. For other abbreviations, see Table 1.

to antigens in the mucosal microflora (32). This response then conditions the mice to respond to TNBS with a massive Th1 response that rapidly inhibits a normal counter-regulatory response.

Type II models, i.e., models that result from an inadequate regulatory response, are exemplified by the SCID-transfer model wherein transfer of naive CD45R<sup>hi</sup> T cells leads to colitis, whereas transfer of both naive and memory (CD45R<sup>low</sup>) T cells does not (73). In this model, as described more fully below, the memory cell population contains regulatory cells so that transfer of only naive cells leads to an inadequate regulatory response and colitis. A second type II model that results from inadequate regulation is that seen in mice bearing a dominant-negative TGF- $\beta$  RII chain (under a CD4+ promotor) that abrogates TGF- $\beta$  signaling (74, 75). Here, regulatory cells are present, but they cannot function adequately because their intended targets are “blind” to their signals.

In the following detailed review of various models, we characterize their basic mechanisms as a type I model (faulty effector cell function) or as a type II model (inadequate regulatory cell function), and in Table 2 we have categorized most of the models on this basis.

**Figure 1** (*upper panel*) The normal mucosal immune system displays a balanced effector T cell response (Th1 or Th2) and regulatory T cell response (Th3 or Tr1). (*lower panels*) The abnormal mucosal immune system displays an unbalanced response consisting of either excessive effector cell response (type I models) or inadequate regulatory cell response (type II models).

## The Role of Mucosal Microflora in the Induction of Mucosal Inflammation

Regardless of whether the experimental mucosal inflammation is type I or type II in character as defined above, the driving force of the inflammation is the non-pathogenic commensal organism resident on the mucosal surface, the mucosal microflora. This is supported by the data in Table 3, which shows that, with perhaps one or two exceptions, mice developing disease in a specific pathogen-free or conventional environment do not do so in a germ-free environment, and in most instances disease is ameliorated when the mice are treated with antibiotics that rid the mucosa of certain classes of organisms (reviewed in 21, 76–85).

Exceptions to this consistent pattern are informative. The first exception is the IL-2-deficient mouse, which develops severe and aggressive gastritis, duodenitis, and colitis under conventional conditions but only nonfatal, mild, focal, and non-proliferative gastrointestinal inflammation under germ-free conditions (80, 81, 86). In addition, these mice develop peri-portal hepatic inflammation, anemia, and generalized lymphoid hyperplasia, which is not ameliorated by the presence of a germ-free state. Thus, this exception to the rule can be explained if we assume that autoimmune inflammation against nonmucosal self-antigens is a component of IL-2 deficiency disease. The second exception is the induced colitis known as dextran sulfate colitis or DSS colitis. This model of colitis can also be observed under germ-free conditions (at least in some studies), although it is ameliorated by antibiotic treatment (21, 83, 84). This can be explained by the fact that this

**TABLE 3** Colitis in models of mucosal inflammation in germ-free vs. specific pathogen-free (SPF) or conventional conditions

	SPF	Germ-free	Antibiotic treatment
SCID-transfer colitis	+	0	<sup>c</sup>
IL-2 deficiency colitis	++	Mild, focal <sup>a</sup>	?
IL-10 deficiency colitis	++	0	
Tgε26	+	0	?
TCR-α chain colitis	+	0	?
SAMP-1/Yit mice	+	0	?
DSS colitis	+	0/+ <sup>b</sup>	
Carageenan colitis	+	0	
Indomethacin colitis	+	0	

<sup>a</sup>See references 80, 81, 86.

<sup>b</sup>See references 21, 83, 84.

<sup>c</sup>Decreased colitis.

Abbreviations: SCID, xx; TCR, T cell receptor; SAMP, xx; DSS, dextran sulfate sodium.

is a colitis caused primarily by direct activation of macrophages by a physical agent (DSS), and T cell responses appear to be superimposed phenomena that can aggravate but are not essential to the inflammation. Thus, this exception to the rule is due to the fact that the colitis is at least in part driven by nonimmune factors.

Additional and more direct evidence that mucosal microflora drive mucosal inflammation in models of mucosal inflammation comes from studies showing that mouse lamina propria T cells are usually unresponsive to their own microbial flora (with respect to either proliferation or cytokine production) but are responsive to the microflora of other individuals even if the other individual is a mouse of the same strain (87, 88). Thus, quite remarkably, oral tolerance to “self flora” appears to be every bit as specific as tolerance to “self antigens.” A related observation is that mice with TNBS colitis lose their nonresponsiveness to their own flora and regain it when the colitis resolves (88). This suggests that the colitis is at least in part driven by the antigen in the microflora, either as a result of cross-reactivity to TNBS or because with the onset of colitis, tolerance to many microflora antigens is lost. Those latter possibilities are supported by the finding that systemic immunization of IL-2-deficient mice with TNP-KLH or other TNP-substituted proteins produces rapid onset of colitis that is identical to the spontaneous colitis occurring in these mice (89). It is important to note that such loss of tolerance to self flora is also a feature of human inflammatory bowel disease (IBD), a fact suggesting that the human disease is also due to loss of tolerance to self microflora (87, 88, 90).

A third kind of evidence supporting the fact that antigens in the normal microbial flora drive mucosal inflammation comes from an extensive series of studies of the spontaneous colitis occurring in an LPS-nonresponsive C<sub>3</sub>H/HeJ mouse substrain (called C<sub>3</sub>H/HeJBir mice), which is discussed more fully below. Suffice it to say here that CD4+ T cells stimulated *in vitro* by lysates of resident bacteria can transfer disease to naive disease-free recipients (91, 92). Similarly, studies of mice bearing a STAT4 transgene show that *in vitro* exposure of T cells from mice with an increased propensity to undergo Th1 T cell differentiation to autologous microfloral antigens induces in these T cells the capacity to cause a Th1 colitis in SCID recipients (72). Together, these studies provide a direct demonstration that T cells specific for mucosal microflora act as effector cells in models of mucosal inflammation. It should be noted, however, that whereas effector cells inducing colitis can be stimulated by antigens in the mucosal microflora, regulatory cells can also be so stimulated. This is shown in additional studies of C<sub>3</sub>H/HeJBir mice in which it was found that cell lines producing IL-10 could also be derived from these mice, which upon co-transfer with effector cells prevented development of colitis (reviewed in 93). From these studies and other studies below it is evident that the mucosal microflora can also induce regulatory cells, and it is really the loss of balance between induction of effector and regulatory cells that defines when disease occurs.

The fact that the mucosal microflora is the major driving force in experimental inflammatory disease should not be taken to imply that all bacterial antigens take part in the disease process or even that the same antigens are necessarily implicated

in a number of different models of inflammation. This is evident from the studies of the aforementioned C<sub>3</sub>H/HeJBir model showing that relatively few antigens within the large antigenic pool of the mucosal microflora are actually found to stimulate B cells or T cells during the course of the disease (92). One must hasten to add, however, that while the number of stimulating antigens was low relative to the total number of antigens present, it was still considerable (see below). A similar situation is observed in the SCID-transfer and TCR- $\alpha$  chain-deficient mouse models (34, 94). In the latter, T cells with aberrant T cell receptors (TCRs) exhibit restricted T cell clonality in conjunction with a common (public) motif in the CDR3 region of the TCR (33, 34). However, in this case the aberrant TCR probably dictates a limited ability to recognize the full set of antigens and thus, may exaggerate the narrowness of the antigenic repertoire recognized by the colitic mice. Similar considerations apply to human patients with IBD who also exhibit restricted T cell clonality and evidence of public motifs among the cells present in lesional tissues (95). Thus, in some patients with IBD there is evidence that the restricted T cell clonality reflecting the presence of a limited group of related stimulatory antigens may be involved in disease pathogenesis. However, in the majority of patients the data are more consistent with a broader T cell response that is characterized by the presence of private motifs that vary from individual to individual.

The restricted yet variable nature of the antigens of the mucosal microflora capable of evoking mucosal inflammation does not conflict with the fact that mono-association of HLA-B27 transgenic rats or mice with IL-10 deficiency and TCR- $\alpha$  chain deficiency with *Bacteroides vulgatus* can lead to colitis (76, 77, 96, 97). First, *B. vulgatus* is likely to be one among many organisms that can induce disease. Second, the effects of *B. vulgatus* mono-association may relate to its ability to synergize with other Enterobacteriaceae in causing infection or to augment internalization of selected strains of bacteria (98). Similarly, *Helicobacter hepaticus* infection causes disease in IL-10-deficient mice under some animal room conditions but not others (99, 100). In addition, in one study microflora that included *H. hepaticus* caused colitis in Rag-2-deficient mice but not Rag-2-deficient mice also deficient in IL-7 or Rag-2-deficient mice treated with IL-10 (101). Because overproduction of IL-7 by epithelial cells is a cause of colitis in another model (102), these results suggest that the ability of an organism to cause colitis may depend on its ability to directly stimulate a particular cytokine pattern in the mouse host and thereby cause undue activation of certain cell populations, such as macrophage populations.

Studies involving antibiotic treatment of murine models of inflammation with antibiotic also attest to the fact that many bacterial species are capable of promoting inflammation. Thus, whereas either ciprofloxacin or a combination of neomycin and metronizadole could prevent colitis in IL-10-deficient mice, only the combined antibiotic regimen was successful as a treatment of the colitis (85). Similarly, combinations of vancomycin plus imipenem were necessary to treat disease in IL-10-deficient mice, DSS-treated mice, and HLA-B27 transgenic rats, and other

combinations of broad spectrum antibiotics were necessary to treat mice with TNBS colitis (21, 76, 77, 103).

In line with the above discussion of effector and regulatory cells stimulated by mucosal microflora, certain organisms appear to be particularly involved in the induction of mucosal inflammation, presumably owing to their capacity to stimulate effector cells, and other organisms may have a special capacity to quell inflammation via an enhanced capacity to stimulate regulatory cells. Evidence in favor of this concept is that introduction of *Lactobacillus* species into the mucosal environment of IL-10-deficient mice prevents the development of colitis of the mice under specific pathogen-free conditions (104). Additional evidence comes from the observation that whereas colitis in mice with *Mdr1a* deficiency is worsened by infection with *Helicobacter bili*, it is ameliorated by infection with *H. hepaticus* (105). Finally, spontaneous colitis in the SAMP1/Yit mouse is more severe in a pathogen-free environment than in a conventional environment (37). This finding is relevant to human IBD because it is possible that the increase in the incidence of IBD observed in developed countries may be due to the fact that exposure to organisms that could ameliorate potential inflammation is decreased in these countries. This view is consistent with recent studies by Dalwadi et al. showing that a superantigen (called I2) derived from *Pseudomonus* species is associated with Crohn's disease lesions and induces the regulatory cytokine IL-10 in vitro (106). Overall, these considerations make it likely that the presumed nonresponsiveness to mucosal flora may be more apparent than real in that normal organisms are responding to antigens in the mucosal microflora but only in the negative sense of inducing regulatory cells or the production of organism-specific IgA antibodies that regulate colonization and translocation.

A final point of some interest is that the bacterial flora present in a particular niche in the intestine may have increased importance in eliciting inflammation in an animal model. Thus, creation of a cecal self-filling blind loop in HLA-B27 transgenic rats leads to proliferation of anaerobic bacteria, especially *Bacteroides* species and a more severe transmural cecal inflammation (103). Moreover, exclusion of the cecum leads to reduced gastric inflammation. Finally, in TCR- $\alpha$  chain-deficient mice, early removal of the tip of the cecum containing a large lymphoid aggregate leads to attenuation of subsequent colitis (107). Thus, it is possible that bacteria occupying a particular area of the intestine are of increased importance in generating effector cells that ultimately cause disease in all parts of the intestine.

In summary, there can be no question from the foregoing discussion that the mucosal microflora play a critical role in models of mucosal inflammation by providing the major stimulus for the induction of effector T cells that cause the inflammation. This being said, it is also apparent that no single bacterial antigen has yet been shown to be responsible for this stimulation, although clearly some bacteria may be more important than others in this respect. Thus, whereas a continued search for a particular organism and/or antigen that causes IBD remains an important goal of some IBD investigators, the advent of models of mucosal inflammation that collectively show that mucosal inflammation is associated with

inherent immune defects in the face of an unaltered flora indicates that this goal may prove futile.

## Genetic Factors in Models of Mucosal Inflammation

**STUDIES IN MOUSE MODELS** The possible genetic factors underlying models of mucosal inflammation have only recently received attention. Such factors could conceivably be operative both in models of spontaneous or induced colitis in mice strains with no known underlying genetic defects, or in mice with a known gene deletion or over-expression. In the latter case the genetic factor could conceivably influence the expression of the known gene defect. Evidence of such factors has been shown in relation to DSS colitis, in which it has been demonstrated that different mouse strains have different susceptibilities to disease (108, 109). Not surprisingly, strains in which mucosal inflammation has occurred spontaneously, such as the C<sub>3</sub>H/HeJBir mouse, have proved highly susceptible to DSS colitis, as did autoimmune-prone NOD mice of various types (108). Interestingly, Non-/LtJ mice were quite resistant to DSS colitis even though NON mice are congenic with NOD with respect to MHC. This lack of involvement of MHC genes in this form of colitis, if generalizable, is consistent with the view that no single antigen or set of antigens is involved in inducing experimental mucosal inflammation.

In a second published study of genetic factors, a genome-wide search for quantitative trait loci (QTL) for susceptibility to DSS colitis in susceptible C<sub>3</sub>H/HeJ mice was conducted (110). In this study the C<sub>3</sub>H/HeJ mice were crossed with partially resistant C57BL/6 mice and strain-specific genetic areas associated with occurrence of colitis in their F<sub>2</sub> progeny was determined. A number of QTLs were identified including those on chromosomes 1, 2, 5, and 18. In addition, several resistance loci were identified in susceptible NOD/Lt strain mice carrying resistance alleles from either B6 on chromosome 2 or from NON/Lt on chromosome 9. Thus, the genetic factors present in DSS colitis were highly complex.

A third study of genetic factors in mouse models examined genetic factors controlling disease severity in IL-10-deficient mice (111). Here, IL-10-deficient mice on a C<sub>3</sub>H/HeJBir background manifested severe colitis when intercrossed with IL-10-deficient mice on a C57BL/6 background that manifested mild colitis; this was done to determine inheritance of disease in the F<sub>2</sub> generation and thus to identify QTLs. A C<sub>3</sub>H-derived colitogenic locus was found on chromosome 3 in two separate studies. This locus interacts in a complex fashion with other loci including a BL6-derived QTL on chromosome 18, a C<sub>3</sub>H-derived QTL on chromosome 8 for cecal lesions, and a C<sub>3</sub>H-derived disease QTL on chromosome 3, chromosome 9, and chromosome 19. These crosses thus found colitogenic susceptibility modifier genes that interact with IL-10 deficiency to cause more severe disease.

Finally, an as yet unpublished study of genetic factors in TNBS colitis using susceptible SJL/J mice and resistant C57BL/6 mice subjected to a similar genome-wide search revealed loci on chromosomes 9 and 11 (G. Bouma & W. Strober, unpublished observations). These findings parallel those derived from a recent

study of SAMP1/Yit mice that also revealed the existence of a locus on chromosome 9 (112). Thus, a QTL in chromosome 9 may well harbor an important gene involved in susceptibility to colitis both in mouse models of inflammation and in humans. The QTL in chromosome 11 in the study of TNBS colitis is also of interest because the tendency of this strain of mice to manifest high IL-12 responses maps to the same region. Thus, the possibility emerges that a gene controlling IL-12 responses is an important susceptibility gene in a model of colitis as well as in IBD.

**STUDIES IN HUMANS WITH IBD** One area of research into mucosal inflammation in which studies of humans with IBD can inform us about murine models of inflammation rather than vice versa is the area of genetic factors operating in these abnormalities. Two developments in the study of IBD are relevant. The first is that large-scale genome-wide searches conducted in families containing multiple members with IBD have led to the identification of 12 chromosomal loci associated with the occurrence of disease (113). In some but not all cases these loci have been confirmed by two or more independent studies and thus are genomic areas where disease genes can eventually be found. The finding that human IBD is a multigenic disease as implied in these human studies has relevance to the murine models, as it indicates that multiple genes are involved in the murine models even when the latter is due to a known genetic defect. This explains the fact that the expression of disease in, for example, Gi2a-deficient mice varies greatly with the strain of mouse bearing this defect. Finally, it is important to note that studies of susceptibility and resistance genes for murine mucosal inflammation, such as those discussed above, can greatly facilitate this search for disease genes in humans because the location of identified murine genes can ultimately be linked to syntenic genes in humans.

The second development in the study of IBD is that the gene located in the most well established of the above loci, that in IBD-1, has recently been identified by two independent groups using two independent techniques (114, 115). These groups have shown that a gene encoding the protein present in macrophages and known as NOD-2 is a disease gene in Crohn's disease; some 10–20% of individuals with the disease have mutations in NOD-2 and those that are homozygous for a mutated gene will invariably develop the disease. The function of the NOD-2 gene is poorly understood and thus its relation to the pathogenesis of IBD is essentially unknown. As reviewed by Beutler (116), some hints as to its function come from a knowledge of its structure: NOD-2 contains on one end a leucine-rich region where most of the mutations have been found and on the other end a caspase recruitment domain. Leucine-rich regions are thought to be binding regions and are found in toll-like receptors (TLRs). Thus, one possibility for the function of NOD-2 is that it interacts with ligands of TLRs (LPS and other bacterial products) that have gained entrance to the interior of the cell and then activate the NF- $\kappa$ B pathway through RICK, a protein known to bind to NOD-1, a close homologue of NOD-2. In this scenario, mutations of NOD-2 in Crohn's disease are gain-of-function mutations that lead to increased NF- $\kappa$ B activation and inflammation. This would imply that

processes involved in natural immunity (i.e., interactions of bacterial products with substances similar to those on TLRs) rather than adoptive immunity may play an important role in the initiation of Crohn's disease. An alternative function of NOD-2 relates to the presence of the caspase recruitment domain and the possibility that NOD-2 is involved in caspase activation and apoptosis. One could propose that the mutations are loss-of-function mutations that lead to decreased apoptosis and thus to the persistence of cells that produce inflammatory cytokines. At the moment, neither of these possibilities is supported by either *in vitro* or *in vivo* evidence, and further studies are necessary to decide their validity or, indeed, the validity of other possibilities.

Finally, with respect to the murine models of mucosal inflammation, none have yet been identified that appear to have NOD-2 mutations. Nevertheless, it is still possible that one or more of the models with no identifiable cause such as a SAMP1/Yit model can be due to a NOD-2 mutation, particularly because this model so closely resembles human Crohn's disease (see discussion below). In addition, it is possible that, as implied above, a NOD-2 mutation acts as a contributing "background" abnormality determining susceptibility to mucosal inflammation primarily owing to another defect.

## Th1 MODELS OF MUCOSAL INFLAMMATION

By far the most common immunologic mechanisms leading to a model of mucosal inflammation are those involving a dysregulation of the Th1 T cell pathway. As already mentioned, the most important reason for this Th1 bias is that conditions in the mucosal environment, particularly the ubiquity of substances that induce IL-12, favor excessive Th1 response over excessive Th2 response if and when there is an imbalance in mucosal immune homeostasis. In general, the nature of the inflammation at both the macroscopic and microscopic levels in Th1 models is most closely related to Crohn's disease, and indeed, this disease has quite clearly been shown to be due to a Th1 T cell disturbance (or a set of disturbances).

Of the various Th1 models, two have been studied most intensively and have yielded insightful information. We discuss these models in some detail.

### TNBS Colitis

Hapten-induced colitis [trinitrobenzene sulfonic acid (TNBS)–colitis] is an important model of mucosal inflammation because it allows for the study of early or initiating events in the development of a mucosal inflammation and because it allows analysis of the relation of the response to a specific antigen (a hapten) to the overall mucosal immune response leading to colonic inflammation. Whereas this model has been the object of study for over two decades (117, 118), it was not until 1995 when Neurath et al. showed that TNBS administered per rectum (in the presence of ethanol) to SJL/J mice resulted in a transmural infiltrative

disease limited to the colon and owing to an IL-12-driven, Th1-mediated response (32). The latter was definitively demonstrated by the fact that treatment of mice with only a single dose of anti-IL-12 antibody results in complete prevention of TNBS colitis or, in the case of mice with preexisting and ongoing disease, in complete and rapid disappearance of the inflammatory lesion (32). In addition, it was shown that, in common with the colonic inflammation seen in IL-2-deficient mice or in several other models of colonic inflammation, the disease could be prevented by administration of anti-CD40L (CD154), indicating that the Th1 response driving this Th1-mediated inflammation was based on CD40L-CD40 interactions (91, 119–121).

In subsequent studies of the role of the various IL-12-induced Th1 cytokines participating in the pathogenesis of TNBS colitis, it has been shown that the role of TNF- $\alpha$  is surprisingly important. In particular, TNBS could not be induced in TNF- $\alpha$ -deficient mice and is far more severe in mice that over-express this inflammatory cytokine (122). One possible explanation of these findings is that TNF- $\alpha$  is necessary for both the initiation and persistence of the Th1 response, possibly by acting as a proximal cofactor for IL-12 or IL-18 production.

The dramatic effect of anti-IL-12 antibody administration on TNBS colitis (and as subsequently shown, on other murine models of colitis) can be linked to the observation that such administration is associated with increased numbers of TUNEL-positive cells in lamina propria tissues and in dispersed cell populations (123). This, plus administration of Fas-Fc to mice undergoing treatment with anti-IL-12, strongly supports the idea that anti-IL-12 treatment leads to Fas-mediated Th1 T cell apoptosis and that TNBS colitis is rapidly responsive to anti-IL-12 because the latter leads to the death of the Th1 T cells inducing the colitis.

One of the major insights derived from the study of TNBS colitis is that regulatory mechanisms inherent in the mucosal immune responses can prevent the development of colitis. This was shown by the fact that whereas intrarectal administration of TNBS led to colonic disease, oral administration of TNBS in the form of TNP-haptenated colonic protein (TNP-CP) prevented colitis induced by intrarectal TNBS administration (124, 125). In addition, it was shown that the preventive effect was due to the induction of regulatory cells producing TGF- $\beta$ , because TNP-CP feeding led to the appearance of TGF- $\beta$ -producing cells in the lamina propria, and coadministration of anti-TGF- $\beta$  antibody to mice fed TNP-CP abrogated the protective effect. This TGF- $\beta$ -mediated protection is due to the induction of oral tolerance in the face of an induced mucosal inflammation and indicates that immune responses resulting in inflammation of the mucosa are as subject to mucosal regulatory effects as the response resulting from the feeding of protein antigens (24, 67–69). On this basis, it is reasonable to attribute the induction of TNBS colitis in SJL/J mice by intrarectal TNBS administration alone to the fact that such administration engenders a mucosal Th1 T cell response that is not balanced by the prompt appearance of a regulatory response. We return to the possible reason why this is so after we discuss the role of mucosal microflora in TNBS colitis.

In other models of mucosal inflammation, most notably that seen in IL-10-deficient mice, IL-10 rather than TGF- $\beta$  appears to be the major cytokine-mediating regulation. In recent, as yet unpublished, studies of the relation between TGF- $\beta$  and IL-10 production in TNBS colitis involving administration of anti-TGF- $\beta$  and anti-IL-10 as well as adoptive transfer of regulatory cell populations, it was shown that TGF- $\beta$  appears to be the major regulatory cytokine but that IL-10 is necessary for the maintenance and/or the effectiveness of the TGF- $\beta$  response (125a). Thus, in the key experiment of these studies it was shown that whereas mice fed TNP-CP and then given TNBS per rectum to induce colitis were protected from colitis, administration of anti-IL-10 after feedings prevented protection and reduced both IL-10 and TGF- $\beta$  responses; however, administration of anti-TGF- $\beta$  prevented protection and reduced TGF- $\beta$  responses but not IL-10 response. Thus, TGF- $\beta$  levels were more closely associated with counter-regulation than were IL-10 levels.

In recent studies taking advantage of the potent capacity of TGF- $\beta$  regulatory cells to ameliorate TNBS colitis, DNA encoding active TGF- $\beta$  was administered to mice intranasally to induce genetically engineered T cells producing TGF- $\beta$  in vivo (29). Indeed, following such treatment, T cells and macrophages producing TGF- $\beta$  were subsequently found in lamina propria and spleen, where they acted to prevent induction of and treat TNBS colitis. Interestingly, the induction of such regulatory cells was associated with production of high levels of IL-10, which also contributed to the regulatory effect. These studies open the door to the possibility that gene therapy with genes encoding regulatory cytokines will become a viable form of treatment of Th1 mucosal inflammation.

Whereas TNBS (or more specifically the TNP epitope) may be the main antigenic stimulus that drives the Th1 responses in TNBS colitis, it is likely that other antigenic determinants present in the mucosal microflora also contribute to the immune response driving this disease. This view stems from evidence reviewed above, which shows that mice with TNBS colitis react to their own microflora and that such reactivity disappears with anti-IL-12 treatment (87, 88, 90). Reactivity to mucosal microflora also relates to TNBS colitis in a way that bears on genetic factors in this model. Recall that TNBS administered per rectum to induce colitis is administered with ethanol, a substance that disrupts the mucosal barrier and thus, as an initial event, causes increased exposure of the mucosal immune system to mucosal microflora. In SJL/J mice that are susceptible to colitis there is evidence that such exposure leads to a high IL-12 response, and it is reasonable to suppose that this sets in motion a massive Th1 response to TNBS that precludes a concomitant regulatory TGF- $\beta$  response. In contrast, administration of TNP-CP by mouth (in the absence of ethanol) does not lead to an initial IL-12 response, and thus a normal mucosal response replete with a regulatory component ensues. Carrying this concept one step further, one might postulate that mouse strains susceptible to TNBS colitis are precisely those that mount high IL-12 when exposed to mucosal microflora. Evidence that this is the case comes from the study of susceptibility loci in SJL/J mice mentioned above, showing that two such loci controlling TNBS

colitis can be identified, and one of these is similar if not identical to that associated with high IL-12 responsiveness (G. Bouma, W. Strober, submitted for publication).

In summary, TNBS colitis in SJL/J mice is an immunologically mediated colitis that results from the rapid induction of an IL-12-driven, Th1-mediated response that precludes development of a counter-regulatory TGF- $\beta$  response. As such, this is a type 1 model in that the major driving force is the overactivity of disease-causing effector cells. Emerging evidence suggests that this response is genetically controlled, most probably by genes that regulate the magnitude of an initial IL-12 response to substances in the mucosal microflora.

## The SCID-Transfer Model of Colitis

A second important model of mucosal inflammation is that produced by repletion of SCID or Rag2<sup>-/-</sup> with either CD45RB<sup>hi</sup> T cells (naive T cells) or with a combination of CD45RB<sup>hi</sup> T cells and CD45RB<sup>lo</sup> T cells (mature T cells) (73, 126, 127). In the former case repletion leads in 3–5 weeks to severe colitis, whereas in the latter case no inflammation occurs. Herein lies the power of the model: One can immediately identify two cell populations, one a source of effector cells and the other a source of regulatory cells, and one can conduct analyses of each population to identify the cells necessary for each type of function.

In initial studies of this model it was found that the inflammation was due to a Th1-mediated T cell response, also driven by IL-12 and mediated by IFN- $\gamma$  (128, 129). In this instance whereas the colitis was less effectively inhibited by anti-TNF- $\alpha$  treatment than TNBS colitis, cells from STAT4-deficient mice still gave rise to disease, perhaps because of their continued ability to produce TNF- $\alpha$  (130).

Whereas CD45RB<sup>hi</sup> cells populate the small intestine as well as the large intestine of SCID recipients undergoing cell transfer, inflammation is limited to the colon. This immediately suggested that organisms endogenous to the colon provide the antigenic stimulus for the mucosal inflammation in this model. Evidence in support of this concept came from studies of the SCID-transfer model that showed that transfer of cells to mice reared in a “near gnotobiotic” environment (rather than the specific pathogen-free environment of the mice in the original studies) manifested greatly reduced levels of inflammation (94). In addition, CD4<sup>+</sup> T cells from colitic mice proliferated and produced Th1 cytokines in response to antigen presenting cells pulsed with fecal extracts of normal but not germ-free mice (94).

If indeed mucosal microflora drive effector cells in SCID-transfer colitis, the number of stimulating antigens in the microflora is circumscribed, because colitic mice contain populations of cells with restricted T cell receptor (TCR) diversity and expression of particular CDR3 sequences (131). It should be noted, however, that the selected TCRs differ from mouse to mouse despite MHC class II identity, indicating that the number of potentially stimulating antigens may be considerable. Finally, the expanded clones were widely dispersed in lymphoid tissue and were

detected early (prior to development of overt colitis), indicating that stimulation of these clones was an early event in disease pathogenesis. Overall, these data are compatible with the view that cell activation in SCID-transfer colitis is an antigen-driven event occurring as a result of inappropriate responsiveness to antigens in the mucosal microflora.

As noted above, the SCID-transfer model is particularly useful for the study of regulatory cells in mucosal inflammation. In an early study of such regulation it was shown that the protective effect of CD45RB<sup>lo</sup> T cells was not due to the secretion of IL-4 because the cells that mediated the protection could be obtained from IL-4-deficient mice and protection occurred in spite of repeated administration of anti-IL-4; however, it was due to secretion of TGF- $\beta$  because in this case repeated anti-TGF- $\beta$  administration reversed protection (132). In more recent studies of the relationship of TGF- $\beta$  and the regulation of SCID-transfer colitis it was shown that the regulatory cells were CD25<sup>+</sup> T cells because CD45RB<sup>lo</sup> cells depleted of CD25<sup>+</sup> T cells were unable to prevent colitis (133). In addition, evidence was presented that the protection afforded by CD25<sup>+</sup> T cells was abolished by co-administration of anti-CTLA-4 and anti-TGF- $\beta$  antibodies, indicating that this subset required stimulation via CTLA-4 and either produced TGF- $\beta$  itself or induced such secretion in other cells. That the former possibility is correct is supported by recent studies by Nakamura et al., who showed that CD25<sup>+</sup> T cells produce TGF- $\beta$  when stimulated by anti-CD3 antibody under crosslinking conditions and costimulated with anti-CD28 or anti-CTLA-4 (59). Thus, the picture that emerges is that CD25<sup>+</sup> T cells in the CD45RB<sup>lo</sup> T cell populations secrete TGF- $\beta$  in a CTLA-4-dependent fashion to mediate suppression of colitis. Parenthetically, recent evidence suggests that CD25<sup>+</sup> cells express surface TGF- $\beta$  in the form of a latent (inactive) protein associated with latency-associated protein. This surface TGF- $\beta$  may be responsible for CD25<sup>+</sup> T cell suppression mediated by cell-cell contact, i.e., the form of suppression exerted under suboptimal stimulation and in the absence of overt inflammation.

A final point to be made about the regulation of SCID-transfer colitis by CD25<sup>+</sup> T cells relates to the fact that, as shown originally by Sakaguchi et al. (134), such cells develop in the thymus and, as recently shown by Bensinger et al. (135), are dependent on the presence of MHC class II-positive cortical epithelial cells for their intrathymic development. Thus, it is not surprising that CD25<sup>+</sup> cells from MHC class II-deficient mice neither act as suppressor cells in *in vitro* assays nor suppress colitis when injected together with CD4<sup>+</sup> CD45RB<sup>hi</sup> cells into Rag-2-deficient recipients. This evidence that CD25<sup>+</sup> cells regulating colitis can originate in the thymus should not be taken to mean that this is the only site of development of these regulatory cells. It remains possible (albeit unproven) that such cells also develop, or at least undergo expansion, in the mucosal tissues.

IL-10, no less than TGF- $\beta$ , has also been implicated in the regulation of SCID colitis. Initial evidence for this came from a study showing that CD45RB<sup>hi</sup> T cells do not cause colitis if obtained from IL-10 transgenic mice (136). In further studies, Groux et al. showed first that T cell clones expanded *in vitro* in the presence

of IL-10 produce high levels of IL-10 (and IL-5) and, in some cases, TGF- $\beta$  as well (55). They then showed that these clones suppress T cell response *in vitro* and, more importantly, suppress SCID-transfer colitis when administered in place of CD45RB<sup>lo</sup> T cells. These studies thus defined a new class of regulatory cells (called Tr1 cells) that mediate immune suppression mainly via IL-10. It should be noted, however, that these cells also produce TGF- $\beta$  and thus the relevant suppressor cytokine is uncertain. A final series of studies relative to IL-10 and regulation of SCID-transfer colitis showed that CD45RB<sup>lo</sup> T cells obtained from IL-10-deficient mice do not prevent colitis when administered with CD45RB<sup>hi</sup> T cells; similarly, treatment of SCID mice administered both CD45RB<sup>hi</sup> and CD45RB<sup>lo</sup> T cells with anti-IL-10 receptor led to the development of colitis (137). These studies thus show that IL-10 is necessary for protection against colitis even if it is not sufficient.

Taken together, the above studies show quite definitively that IL-10 and TGF- $\beta$  are important regulatory cytokines in SCID-transfer colitis. The question arises here, even more than in the case of TNBS colitis, as to how these regulatory cytokines interact to bring about regulation. To date, no studies addressing this question in the context of SCID-transfer colitis have appeared; however, based on the data derived from the TNBS colitis model we would suggest that TGF- $\beta$  is the major suppressor cytokine and that IL-10 is needed to facilitate TGF- $\beta$  secretion and/or activity.

Yet another cell type contributing to regulation of SCID-transfer colitis is the NK cell. This is supported by the fact that transfer of CD45RB<sup>lo</sup> T cells into NK cell-depleted recipients results in more severe colitis (60). Such regulatory effects, as noted above, are also seen in other models of mucosal inflammation and occur via an unknown mechanism. However, it is known that this form of regulation is distinct from that mediated by CD45RB<sup>lo</sup> cells inasmuch as depletion of the latter of NK cells does not eliminate their regulatory effect. One possible but highly speculative explanation is that NK cells lyse activated effector cells or APCs inducing the effector cells. This concept is supported by the fact that NK cells from perforin-deficient mice have no regulatory effects (60).

A final point to emerge from studies of the SCID-transfer model relates to the recent observation that colitic mice have greatly increased numbers of CD134<sup>+</sup> (OX40<sup>+</sup>) dendritic cells in their mesenteric lymph nodes and that administration of anti-CD134L antibody leads to reversal of colitis (as it does in other models) (43, 138). It is likely that these mesenteric lymph node cells originate in the inflamed lamina propria and then migrate to the draining lymph node where they provide inductive signals to effector T cells about to migrate into lesional tissues. This would imply that much of the antigen presentation necessary for the development of mucosal lesions goes on in regional lymph nodes, rather than in the inflamed tissue itself.

In summary, the SCID-transfer model is a model of mucosal inflammation that allows one to separate effector and regulatory T cell functions mediating the inflammatory process. Thus, by analysis of both effector cells in CD45RB<sup>hi</sup>

T cell populations and regulatory cells in CD45RB<sup>lo</sup> T cell populations, it has been possible to clearly demonstrate the interplay between various cell types that determine whether mucosal inflammation will occur. In addition, this model demonstrates that abnormal reactivity to antigen in the mucosal microflora can develop in the absence of a genetic abnormality and does not require initial disruption of the epithelial cell barrier. Rather, the only precondition for the occurrence of colitis is a marked imbalance between effector and regulatory cell populations.

### **Type 1 Models of Colitis: Defects that Directly or Indirectly Affect the Synthesis of Key Cytokines in the Th1 Pathway of T Cell Differentiation**

Joining TNBS colitis as type 1 defects leading to mucosal inflammation are several models whose pathogenesis can be traced to abnormalities that lead to the overproduction of key cytokines in the Th1 T cell differentiation pathway, such as IFN- $\gamma$ , TNF- $\alpha$ , and IL-12.

**COLITIS ASSOCIATED WITH DEFECTS IN THE PRODUCTION OF TRANSCRIPTION FACTORS CONTROLLING IFN- $\gamma$  PRODUCTION** Given the central role of IFN- $\gamma$  in the Th1 responses, it should come as no surprise that molecular defects resulting in IFN- $\gamma$  overproduction can lead to colitis. Two such defects are now known to exist, one affecting T-bet and one affecting STAT4.

As shown by Szabo et al., T-bet is a T-box protein that when over-expressed in T cells programs them for high IFN- $\gamma$  responses and low IL-4 responses, even when the cell is a supposedly "committed" Th2 cell (139). This, plus recent data showing that over-expression of T-bet can lead to IFN- $\gamma$  responses in cells lacking STAT4, has led to the concept that this factor is the molecular switch for Th1 differentiation (140). STAT4, on the other hand, has also been shown to be a necessary factor for Th1 differentiation that probably acts as both a transcription factor for IFN- $\gamma$  and as a factor that maintains Th1 T cell survival (141, 142).

In the relevant studies of colitis associated with a STAT4 abnormality, it has been shown that mice bearing a STAT4 transgene (under a CMV promotor) develop colitis when administered TNP-KLH in Freund's adjuvant, an antigenic stimulus that has no colitogenic effect in normal mice (72). In addition, spleen cells from these mice proliferate when exposed to antigens in their autologous microflora *in vitro*, and T cells thus stimulated induce colitis in SCID recipients. Corresponding studies of T-bet abnormalities have shown that naive T cells from T-bet-deficient mice exhibit a reduced capacity to transfer colitis to SCID mice, whereas, conversely, naive T cells over-expressing T-bet (owing to infection with a T-bet-expressing retrovirus) induce accelerated colitis in SCID mice (M. Neurath, R. Blumberg, L. Glimcher, manuscript submitted). In addition, memory T cells from T-bet-deficient mice exhibit an enhanced capacity to protect SCID mice from colitis when cocultured with naive T cells.

**MUCOSAL INFLAMMATION IN MICE THAT OVER-EXPRESS TNF- $\alpha$ : TNF $\Delta$ ARE MICE** Also in the category of models of inflammation owing to abnormalities of Th1 cytokine production is the model owing to over-expression of TNF- $\alpha$ , the TNF $\Delta$ ARE mouse (36). This model results from a targeted deletion of AU-rich elements located in the 3' untranslated region of the TNF- $\alpha$  gene, which gives rise to dysregulation of the processing of TNF- $\alpha$  mRNA and the overproduction of TNF- $\alpha$  protein. The phenotype of these mice is notable because the mucosal inflammation is mainly located in the terminal ileum and only occasionally in the proximal colon; in addition, it is remarkably similar to that in Crohn's disease: It is a transmural infiltrative lesion that contains typical granulomata. Of considerable interest, TNF $\Delta$ ARE mice develop arthritis resembling rheumatoid arthritis, as well as mucosal inflammation.

Studies of the immunopathogenesis of disease in TNF $\Delta$ ARE mice indicate that whereas the mucosal inflammation is dependent on the presence of T cells, the joint inflammation is not (36). This, plus the fact that the different TNF receptors are involved in the two kinds of inflammations, indicates that the pathogenesis of inflammation in the two areas is different. As far as the disease in the mucosa is concerned, it is likely that the inflammation is initiated and maintained by substances in the mucosal microflora that can induce TNF- $\alpha$  production (LPS, CpGs, etc.). However, it is important to mention that colitis does not occur in TNF $\Delta$ ARE mice that are also IL-12 p40-deficient (F. Cominelli, personal communication); thus, the inductive process does not appear to involve the direct stimulation of cells by microfloral stimuli, but rather their indirect stimulation via IL-12. It is important to mention here that IL-12 production may be enhanced in TNF $\Delta$ ARE mice because once TNF- $\alpha$  overexpression is initiated, a positive feedback loop between IL-12 and TNF- $\alpha$  is established.

**COLITIS IN Gi2A MICE** Mice deficient in the G protein Gi2 $\alpha$  provide yet another type 1 model in which overproduction of a Th1 cytokine results in colitis. The inflammation in this model is a Th1-mediated colitis with an infiltrative histologic picture similar to other Th1 colitides (143). The basis of this colitis has been elucidated by studies showing that a stimulus that inhibits Gi protein signaling, such as pertussis toxin, enhances splenocyte production of IL-12, TNF- $\alpha$ , and IL-10 in vitro upon culture with *Staphylococcus aureus* Cowan I and CD40L (144). In addition, pertussis toxin-treated BALB/c mice exhibit a healing phenotype when infected with *Leishmania major*, whereas untreated mice of this strain manifest progressive infection. That these findings are relevant to Gi2 $\alpha$  mice was shown by the fact that these mice produce increased amounts of IL-12 and TNF- $\alpha$  when their CD8a+ (lymphoid) dendritic cells are appropriately stimulated. Thus, it is reasonable to conclude that the colitis in Gi2 $\alpha$ -deficient mice is a type 1 colitis owing to the overproduction of IL-12. One can reasonably assume that antigens in the mucosal microflora are the driving force of such overproduction, but this needs to be verified experimentally.

## Type 2 Models of Colitis: Defects in the Production of Proteins that are Directly or Indirectly Involved in Regulation of Mucosal Responses

The mirror image of the previous category of models of mucosal inflammation are those in which there is an abnormality in the synthesis of a regulatory cytokine or a protein that affects the function of a regulatory cytokine. These are thus type 2 models and include colitis owing to IL-10 deficiency or abnormalities of IL-10 signaling and to defects in TGF- $\beta$  function.

**COLITIS OWING TO IL-10 DEFICIENCY OR IL-10 SIGNALING DEFECTS** As initially reported by Kuhn et al., IL-10-deficient mice raised in a specific pathogen-free (SPF) or a conventional environment develop colitis marked by epithelial cell hyperplasia and a transmural inflammation (145). Early on, the disease is due to a Th1 response and is completely ameliorated by anti-IL-12 treatment. Later, however, a Th2 response supervenes and the lesion is no longer treatable with anti-IL-12 (35; A.D. Levine, personal communication). The reason for this change is unclear, but it may relate to the fact that in the absence of IL-10 down-regulation, Th2 responses ultimately prevail. Further studies of the cytokines involved in the colitis of IL-10 deficiency have come from studies in which colitis was induced in SPF mice by infection with *H. hepaticus*. It was shown that whereas IL-12 had to be present for colitis to develop (99), IFN- $\gamma$  or TNF- $\alpha$  did not, as treatment with anti-IFN- $\gamma$  or anti-TNF- $\alpha$  had no effect on the colitis; it is thus apparent that each of these cytokines can mediate colitis in the absence of the other (146). Additional studies showed that treatment with both anti-IFN- $\gamma$  and anti-TNF- $\alpha$  was also not effective in the treatment of colitis, suggesting that in IL-10 deficiency yet other cytokines induced by IL-12 may play effector roles in the absence of both IFN- $\gamma$  and TNF- $\alpha$ .

As in other models, the colitis of IL-10-deficient mice does not develop under germ-free conditions and is thus driven by antigens in the mucosal microflora (93, 99, 100). Recent studies of colitis in SPF mice infected with *H. hepaticus* underscore this fact. Thus, the study mentioned above showed that such infection led to greatly enhanced colitis, but another study showed that such infection led to no more colitis than that ordinarily seen under SPF conditions (99, 100, 146). This suggests that mucosal microflora (such as that present in some mouse holding areas) can influence the development of inflammation even when it is driven by a known pathogen. A final point concerning the role of the mucosal microflora in the colitis of IL-10-deficient mice is that these mice manifest increased intestinal permeability even prior to the development of overt colitis. This change in barrier function may lead to increased contact with or stimulation by antigens in the mucosal microflora and is thus a factor that facilitates the development of the inflammation (66).

The basis of the immunoregulatory defect leading to colitis in IL-10-deficient mice undoubtedly lies in the fact that IL-10 has major suppressive effects on

immune responses, and its absence in an area of the body constantly exposed to antigens leads to inflammation in that area. The negative effects of IL-10 on immune responses is well demonstrated in numerous *in vitro* studies showing that IL-10 inhibits IL-12 and TNF- $\alpha$  production, suppresses costimulatory molecules, and directly inhibits T cell proliferation and/or induces T cell apoptosis (147–150). In addition, *in vivo* studies of IL-10 transgenic mice and the SCID-transfer model discussed above have shown that mature CD45RB<sup>lo</sup> T cells from IL-10 deficient mice are incapable of preventing the development of colitis (137). This finding is underscored by the aforementioned studies in which Tr1 cells that produce IL-10 can substitute for CD45RB<sup>lo</sup> T cells in the prevention of SCID-transfer colitis (55). Taken together, these data provide ample reason to postulate that the colitis of IL-10 deficiency is a prototypic type 2 model of colitis owing to absence of a major regulatory cytokine. The only question that remains with respect to this conclusion is the one discussed previously in relation to TNBS-colitis concerning the relation of TGF- $\beta$  and IL-10 in the regulation of mucosal inflammation. It was mentioned in that context that the evidence now available favors the view that TGF- $\beta$  is the more proximal cytokine suppressor and that the main role of IL-10 is to maintain and facilitate the TGF- $\beta$  suppressor effect. This leads to the supposition that in the colitis of IL-10 deficiency it is not the lack of IL-10 *per se* that leads to inflammation, but rather the lack of an adequate TGF- $\beta$  response that occurs in the absence of IL-10.

Finally, it is important to note that not only IL-10 deficiency can lead to colitis, but so can defects in IL-10 signaling that functionally are equivalent to IL-10 deficiency. This is seen in mice deficient in an “orphan” receptor termed CRF-2, which forms part of the IL-10 receptor, and in mice whose macrophages and neutrophils are deficient in STAT3 expression that exhibit a defect in IL-10 signaling (151, 152).

**COLITIS ASSOCIATED WITH TGF- $\beta$  DEFECTS** In light of the discussion above it is to be expected that deficiency in the production of TGF- $\beta$  should also lead to type 2 models of mucosal inflammation. This was presaged by studies of mice with TGF- $\beta$ 1 deficiency owing to targeting of the TGF- $\beta$ 1 gene, in which it was shown that such mice exhibit widespread inflammation in multiple organs and early death (153, 154). It should be noted, however, that this inflammation is not more prominent in mucosal tissues than in other tissues, possibly because the mice die of widespread autoimmune disease before they have the chance to develop inflammation to “exogenous” mucosal antigens.

A more particular relation of defects in TGF- $\beta$  function to mucosal inflammation occurs in mice with defective TGF- $\beta$  signaling, either only in T cells or only in epithelial cells owing to the presence of transgenes encoding dominant-negative TGF- $\beta$  receptors (TGF- $\beta$ RII) under T cell-specific or epithelial cell-specific promoters, respectively (75, 155). In the former case inflammation develops in the colon and lung and the mice develop autoantibodies and glomerular immune complex deposition. Interestingly, both Th1 and Th2 cytokine production is increased,

probably because TGF- $\beta$  regulates both Th1 and Th2 responses. In the latter case of the epithelial-specific expression of the dominant-negative transgene, the mice develop colitis under conventional conditions and manifest increased susceptibility to the development of dextran sulfate colitis. The development of colitis in these mice suggests that TGF- $\beta$  also regulates epithelial cell function and in its absence the mucosal is more subject to stimulation by antigens in the mucosal flora.

### Colitis in which the Presence of Type 2 Defects Are Traceable to Thymic Dysfunction

Whereas mice with IL-2 deficiency (or IL-2R deficiency) obviously have a very different immunologic defect than bone marrow-reconstituted Tg $\epsilon$ 26 mice, the pathogenesis of the colitis associated with these defects is sufficiently similar to warrant their discussion under the same heading. Thus, in both models the inflammation is an IL-12-driven, Th1-mediated inflammation that depends on CD40L-CD40 interactions and is abrogated by the absence of IL-12 (89, 120, 121, 156). In addition, in both models there is evidence that the underlying abnormality is the defective generation of regulatory cells and that an intrathymic defect is probably responsible for this abnormality (157–159).

**COLITIS IN IL-2-DEFICIENT MICE** Mice with IL-2 deficiency exhibit early development of lymphadenopathy, bone marrow infiltration, and hemolytic anemia indicative of a generalized autoimmune state, which are then followed in surviving mice by the development of a transmural colitis (86). The latter is a T cell-driven event that, as mentioned above, is due to a Th1 response (89). T cells in these mice bear markers of maturity and proliferation that may relate to the presence of an apoptosis defect, presumably occurring because in absence of IL-12 there is deficient activation-induced (Fas-mediated) apoptosis (86, 160). In the absence of IL-2, IL-15 may play a major role in this hyperproliferative state, but this is not supported by the relevant available *in vitro* studies (161). As in other models, it is clear that antigens in the mucosal microflora drive the T cells because disease does not develop under germ-free conditions (81, 86). In addition, there is some evidence that in the absence of IL-2, epithelial cells exert increased antigen-presenting function, which plays a role in T cell activation and cytokine secretion (162).

The pathogenesis of colitis in IL-2-deficient mice has been successfully studied by inducing the rapid onset of colitis by intra-peritoneal injection of TNP-KLH in Freund's adjuvant (89). This maneuver apparently stimulates T cells that cross-react with antigens in the mucosal microflora and mediate disease. Studies in this induced model of mucosal inflammation disclosed that whereas normal mice react to TNP-KLH stimulation with an IL-4/TGF- $\beta$  response, this response is absent in IL-2 deficient mice (89). Furthermore, they showed that concomitant administration of anti-CD3 does elicit an IL-4/TGF- $\beta$  response and prevents development of disease. That such prevention is due to the TGF- $\beta$  response and not the IL-4 response was shown by the fact that the protection obtained with anti-CD3 treatment was abrogated by simultaneous treatment with anti-TGF- $\beta$  but not with

anti-IL-4. Thus, these studies led to the conclusion that the underlying cause of colitis in IL-2 deficiency is a type 2 defect and an inadequate regulatory response (157).

Further studies of immune function of IL-2-deficient mice as it relates to colitis focused on thymic function because it was known that thymic defects contribute to the autoimmunity seen in these mice (159). Again using the TNP-KLH to induce colitis in IL-2 deficient mice, it was shown that this stimulus leads to the appearance of increased numbers of single positive thymocytes in the thymus that displayed a Th1 cytokine secretion profile and transferred colitis to normal mice (159). These studies thus suggest that thymocyte development is defective in IL-2 deficiency and this defect leads to either increased numbers of effector cells capable of mediating either autoimmunity or colitis or to decreased numbers of regulatory cells capable of preventing these phenomena. Given the above role of regulatory cell dysfunction in IL-2-deficient mice, the latter rather than the former is likely to be the more important factor.

Finally, it is important to mention that mice with IL-2R deficiency owing to either  $\alpha$  or  $\beta$  chain gene targeting also develop autoimmunity and colitis (163–165). However, those with IL-2R deficiency owing to  $\gamma$  chain targeting do not, presumably because such mice cannot mount adequate T cell responses to support autoimmunity. IL-2R $\beta$  chain-deficient mice differ somewhat from IL-2-deficient mice in that they display hypergranulopoiesis that crowds out normal marrow elements and leads to massive lymphoid infiltration with granulocytes (164, 165). In addition, they manifest poor responses to antigen, presumably because their cells respond poorly to both IL-2 and IL-15. This raises the question as to which cytokine is driving their autoimmune responses and leads to the possibility that in IL-2-deficient mice neither IL-2 nor IL-15 is necessary to support T cell responses.

**COLITIS IN BONE MARROW-RECONSTITUTED Tg<sub>e</sub>26 MICE** Tg<sub>e</sub>26 mice are mice bearing a transgenic CD3-epsilon chain whose over-expression results in intrathymic T cell and NK cell death probably because of excessive signal transduction during thymic development (158, 166). In addition, they manifest a secondary defect in thymic stromal architecture because the development of the latter depends on the presence of normal thymocytes (167). Fetal mice bearing the transgene can be rescued by transplantation of T cell-depleted normal bone marrow because such transplantation preserves stromal architecture; in contrast, adult mice cannot be thus rescued because by this time the defect in architecture cannot be reversed (158, 167). Thus, whereas bone marrow reconstitution of adult mice leads to repair of lymphoid depletion, the reconstituted mice contain a cell population that has developed in a defective thymic micro-environment.

Mice with the Tg<sub>e</sub>26 defect who are reconstituted with normal bone marrow (reconstituted Tg<sub>e</sub>26 mice) develop an infiltrative colitis similar to that seen in IL-2 deficiency, which is due to an IL-12 driven Th1-mediated response driven by antigens in the mucosal microflora (158, 168). The IL-12 dependency of the inflammation is nicely shown by the fact that reconstitution of the mice with bone marrow from STAT4-deficient mice exhibit a greatly reduced level of disease, as do

mice treated with anti-IL-12 (156). It should be noted, however, that reconstitution of mice with bone marrow from IFN- $\gamma$ -deficient mice only slightly ameliorates the development of colitis, presumably because in this situation, as in the case of TNBS colitis and IL-10 deficiency colitis, TNF- $\alpha$  can induce inflammation in the absence of IFN- $\gamma$  (156).

In studies of the underlying factors leading to colitis in Tg $_{\epsilon}$ 26 mice, cell transfer studies were performed using mice with and without transplantation of syngeneic normal fetal thymus (158). These studies showed first that transfer of nonmucosal cells from Tg $_{\epsilon}$ 26 mice with colitis into untransplanted Tg $_{\epsilon}$ 26 recipients resulted in colitis similar to that in the donor mice. It was then shown that Tg $_{\epsilon}$ 26 mice reconstituted with normal bone marrow and transplanted with syngeneic fetal thymus (from Tg $_{\epsilon}$ 26 mice) did not develop colitis, whereas those only reconstituted with bone marrow developed colitis. Because the transplanted fetal thymus maintained a normal architecture in mice reconstituted with normal marrow, the mice with the transplants could generate a cadre of normal cells that then intermixed with the abnormal cells arising from the abnormal thymus. Thus, these studies suggest that the abnormal cell population causes colitis because it lacks a regulatory cell population and that the colitis is a type 2 colitis.

The above discussion makes it apparent that in IL-2 deficient mice and in bone marrow-reconstituted Tg $_{\epsilon}$ 26 mice abnormal T cell development in the bone marrow is a major factor in the development of colitis. One theoretical difference, however, is that in the induced IL-2 deficiency model studied, the inducing antigen (TNP-KLH) is exogenous, whereas in reconstituted Tg $_{\epsilon}$ 26 no exogenous antigens are introduced. This difference may be more apparent than real, however, because it is possible that mucosally derived antigens normally enter the thymus and affect thymic selection. Another difference is that in IL-2 deficiency the development of colitis may occur solely because of local mucosal dysregulation, whereas in reconstituted Tg $_{\epsilon}$ 26 mice the thymus appears to be more intrinsic to the disease state.

## Miscellaneous Models of Colitis: Th1 Responses Whose Underlying Immunopathogenesis is Not Understood

Several models of mucosal inflammation have been described in which the basis of the mucosal inflammation has not yet been elucidated. In some cases these models may prove to be quite important because they may be due to one or more defects also present in humans with IBD. Two of these models are described in some detail below and the rest are summarized in Table 4.

**COLITIS IN C3H/HeJBir MICE** A substrain of LPS-unresponsive mice [lacking toll-like receptor 4 (TLR-4)] C3H/HeJ mice, termed C3H/HeJBir mice, have been noted to spontaneously develop a colitis centered in the cecum and proximal colon (169). The colitis consists of an transmural inflammation that begins at 3–6 weeks and gradually wanes. It is an IL-12-driven, Th1-mediated inflammation that can be transferred to SCID recipients by CD4+ T cells from the affected mice.

**TABLE 4** Miscellaneous Th1 and Th2 models of mucosal inflammation

Model	Salient/unique immunopathologic features	Type	Proposed mechanism	Reference
TCR transgenic mice with lymphopenia	Similar to SCID-transfer colitis; presence of Tg T cells that cross-react with mucosal antigens in most cases.	Th1, ? Type 2	Failure of intrathymic development of regulatory cells	204
IL-7 transgenic mice	Infiltrative lesion with crypt abscesses and loss of goblet cells; IL-7 over-expression only in involved areas.	Th1, type 1 Epithelial barrier defect?	Defective epithelial barrier function; activation of mucosal macrophages	102
Over-expression of HLA-B27	Inflammation of stomach as well as small and large intestine; joint inflammation	Th2, type 1;	Facilitated presentation of mucosal antigens to mucosal T cells; CD8+ T cell-mediated inflammation?	205, 206
NF- $\kappa$ B defects; "A20" mouse; I $\kappa$ B $\alpha$ -deficiency	Inflammation in multiple organs including intestine	Type 1	Hypersensitivity to NF- $\kappa$ B activators; inability to regulate NF- $\kappa$ B response	207, 208
p50 deficiency	Typhlo-colitis; apparently normal T cell development	Type 2?	Defect in NF- $\kappa$ B pathway	209
Th2 TNBS-colitis	TNBS-colitis in Balb/c mice or C57BL/6 mice with IL-12 deficiency	Th2 colitis; Th1 component?; type 1	TNBS-induced, Th2 response to mucosal antigens in mice oriented to Th2 responses	210, 211
Colitis in Wiskott-Aldrich syndrome protein (WASP) deficiency	Mild immunodeficiency; superficial inflammation reminiscent of UC rather than CD	Th2 colitis; Type 2?	Abnormality of regulatory cell development?	212

Abbreviations: TCR, T cell receptor; TNBS, trinitrobenzene sulfonic acid; UC, ulcerative colitis; CD, Crohn's disease.

The focus of research utilizing this model has been to define the relation of bacterial flora to the induction of disease. A series of studies with this in mind showed that cells from C<sub>3</sub>H/HeJBir mice manifest increased B cell and T cell reactivity to mucosal antigens (92, 170). However, this reactivity was selective and was more or less limited to antigens associated with several species of facultative anaerobes. This corresponded to the fact that T cells in colitic C<sub>3</sub>H/HeJBir mice displayed a skewed V $\beta$  distribution (as do T cells in other models including the SCID-transfer model and the TCR- $\alpha$  chain-deficiency model) (92). It should be noted, however, that although the number of antigens implicated in the response is a small fraction of the total number of antigens, it is nevertheless a large number; thus, it is highly unlikely that the colitis is due to only a very limited number of antigens. A similar situation probably obtains in humans, in which a skewed expression of V $\beta$  has also been seen.

Recent cell transfer studies of the C<sub>3</sub>H/HeJBir model have disclosed that T cell lines driven by antigens in bacterial lysates also transfer colitis to SCID mice (91). This finding is a direct demonstration that antigens in the mucosal microflora can mediate colitis and corroborates data from studies of numerous other models that show this more indirectly. Whereas some of the lines were composed of effector cells secreting IFN- $\gamma$ , others were slow-growing lines producing IL-10 and were presumed to be regulatory cells. Indeed, these cells inhibited Th1 responses both *in vivo* and *in vitro*. Thus, a reiteration of studies performed in the SCID-transfer model showed that these cell lines, when cotransferred with effector cells, could prevent the development of colitis in SCID recipients (93). Interestingly, this preventative effect was reversed by either IL-10 or TGF- $\beta$ , again raising questions about the relationship of these regulatory cytokines. In any case, the presence of regulatory cells in the colitis of C<sub>3</sub>H/HeJBir mice suggests that the underlying defect in these mice is a partial block in the regulatory cells' development and that the inflammation gradually subsides when these cells finally make their appearance.

One issue raised by the occurrence of colitis in a substrain of mice that does not respond to LPS is the role of this stimulant in the regulation of mucosal immune responses. It appears paradoxical that a defect in the capacity to respond to a strong stimulator of the IL-12 response would be associated with colitis. This paradox, however, is resolved by the fact that numerous other substances associated with the mucosal microflora have this capacity as well. Perhaps a more cogent and specific role for LPS in colitis relates to the possibility that this stimulant is necessary for the normal induction of regulatory cells.

**COLITIS IN SAMP1/Yit MICE** SAMP1/Yit mice were originally derived from AKR mice by extensive interbreeding, first to achieve accelerated senescence and then to enhance the development of intestinal inflammation (37). This model is important because the inflammation is remarkably similar to human Crohn's disease in that it is mainly an ileitis rather than a colitis, and at the microscopic level one sees typical granulomata and other features of Crohn's inflammation. Once again the disease is driven by antigens in the mucosal microflora and is a Th1 event because it can be transferred to SCID recipients by T cells producing IFN- $\gamma$  and TNF- $\alpha$  (37, 38). Interestingly, the transferred cells produce a disease similar to that in the donor mice, suggesting that they have a homing pattern governed by their site of origin or are expanded by antigens specifically present in the ileum. The underlying defect in SAMP1/Yit mice is not yet known. However, recent studies showing that epithelial cells in these mice produce increased amounts of chemokines suggest the presence of a type 1 defect (171).

## MODELS OF COLITIS DUE TO EXCESSIVE Th2 T CELL RESPONSES

Experimental colitis mediated by Th2 T cells forms a separate universe of colitides that, as discussed above, are associated with a form of inflammation that differs from that seen in the more predominant Th1 colitides in that it more closely

resembles ulcerative colitis than Crohn's disease (23). This has given rise to the idea that ulcerative colitis is in fact a Th2 T cell-mediated disease, but the evidence for this notion is ambiguous at best. Thus, whereas IL-12/IFN- $\gamma$  production is not increased in ulcerative colitis, neither is IL-4 production, and the inflammation in lesional tissue is not usually characterized by Th2 inflammatory elements such as eosinophils and mast cells. In fact, the only Th2 cytokine reported as increased in ulcerative colitis is IL-5 (39–42), but this increase could be due to the presence of certain types of regulatory cells that produce IL-5 rather than to Th2 effector cells (i.e., Tr1 T cells). At the moment, therefore, it is premature to call ulcerative colitis a Th2 disease, despite its histopathologic relation to Th2 T cell-mediated experimental colitides.

In the following discussion we review the most extensively studied Th2 models; pertinent data about several additional Th2 models is provided in Table 4.

### Colitis in TCR- $\alpha$ Chain-Deficient Mice

The first and perhaps best studied model of murine inflammation owing to a Th2-mediated response was initially reported by Mombaerts et al., who noted that chronic colitis develops in gene targeted mice lacking TCR- $\alpha$  chains (51). These authors also found that TCR- $\beta$  chain-deficient mice develop only very mild colitis, but recently it was shown that this colitis is more marked if CD5 deficiency is also present (172). Finally, they showed that mice with  $\gamma\delta$  chain deficiency do not develop colitis.

The colitis developing in TCR- $\alpha$  chain-deficient mice is relatively superficial and extends to the submucosa only occasionally. It is characterized by the presence of elongated and distorted crypts as well as by the presence of occasional crypt abscesses, but transmural fissures and granulomata are notably absent. Overall the lesion is different from that seen in Th1 models of colitis and resembles ulcerative colitis rather than Crohn's disease. This fits with the fact that affected mice frequently develop circulating anti-neutrophil cytoplasmic antibodies (ANCA) and other antibodies found in ulcerative colitis patients (107, 173).

Initial studies of the cell populations in TCR- $\alpha$  chain-deficient mice revealed that the main cells were  $\gamma\delta$  TCR-bearing T cells, but these were admixed with a small population of alpha-beta+ (dim) TCR-bearing T cells ( $\beta\beta$  TCR T cells), which later proved to be the effector cells responsible for the inflammation (49, 51). In subsequent studies involving treatment of TCR- $\alpha$  chain-deficient mice with anticytokine antibodies and cross-breeding of the mice with various cytokine-deficient mice, it was established that IL-4 and not IFN- $\gamma$  was the effector cytokine, i.e., the colitis was a Th2 colitis (174–176).

The  $\beta\beta$  TCR T cells established as effector T cells in TCR- $\alpha$  chain colitis recognize antigens via a unique TCR composed of  $\beta\beta$  homodimers, and it was therefore not too surprising that they display greatly restricted TCR diversity following the development of colitis (34). Thus, whereas T cells obtained from mice prior to the development of colitis or from mice on a non-disease-producing elemental diet display a wide range of V $\beta$  family usage, those with colitis or on a regular diet

display skewed  $V\beta$  usage marked by  $V\beta_{8.2}$  predominance (34). In addition, it was found in single-strand conformation polymorphism (SSCP) analysis and CDR3 sequencing studies that T cells from colitic mice display mono- or oligoclonality in all T cell subsets, not just the  $V\beta_{8.2}$  T cell subset. Finally, it was shown that T cell subsets expressing  $V\beta_{8.2}$  exhibiting restricted diversity are characterized by a restricted CDR3 length and conservation of a single negatively charged amino acid in the second portion of the CDR3 sequence (33). This type of amino acid sequence is characteristic of clones specific for self-antigens and thus may represent a “germ-line” sequence that is cross-reactive with a variety of normally nonstimulatory environmental (mucosal) antigens, i.e., antigens that do not elicit effector cell responses in the mucosal immune system. This possibility is supported by the fact that cells with the stereotypic TCRs can be expanded by coculture with colonic epithelial cells, which presumably are presenting antigens derived from resident (nonpathogenic) microflora (33). It is also supported by the facts that  $\beta\beta$  TCR T cells display vigorous responses to food antigens and that mice with TCR- $\alpha$  chain deficiency exhibit a heightened capacity to provide helper function for B cells that produce antibodies reacting to food antigens (34, 175). Finally, it is supported by the fact that TCR- $\alpha$  chain-deficient mice fed an elemental diet do not develop disease unless they are mono-infected with certain organisms (such as *B. vulgatus*) that are presumably among the organisms expressing cross-reacting antigens (97).

The above data, considered as a whole, lead to the conclusion that whereas  $\beta\beta$  TCR T cells may have a restricted ability to respond to antigens in general, they do respond to certain normally harmless antigens that then drives the cells to expand and exert effector cell activity causing disease. This raises the question of why these T cells have this propensity, but other T cells normally populating the mucosal tissues do not. One possibility already suggested by the TCR sequence data is that these cells have escaped negative selection in the thymus (or other selection areas existing in the mucosa) and thus represent a cadre of self-reactive cells that cross-react with mucosal antigens. This possibility finds strong support in independent studies showing that  $\beta\beta$  TCR T cells have a tendency to escape negative selection in the thymus (177, 178). A second possibility relates to the fact that, as discussed above, mucosal responses are normally regulated by tolerogenic mechanisms, including the development of  $\alpha\beta$  TCR T cells that produce suppressive cytokines. Thus, it is reasonable to suggest that the abnormal reactivity of  $\beta\beta$  TCR T cells to certain mucosal antigens is due to the fact that regulatory T cells cannot develop within the  $\beta\beta$  TCR T cell population (nor in the accompanying  $\gamma\delta$  TCR T cell population).

A second, interrelated question concerns the reason why  $\beta\beta$  TCR T cell stimulation leads to Th2 responses and not Th1 responses. One possibility alluded to in the general discussion of models is that the course of T cell differentiation depends largely on the nature of the antigen-presenting cell and the cytokine environment of the APC-T cell interaction. In this context it is already known that certain dendritic cells present in the Peyer's patches (CD11c+ dendritic cells) preferentially

secrete IL-10 rather than IL-12 and induce Th2 T cells rather than Th1 T cells during antigen presentation (70, 71). Thus, it is possible that the types of antigens that stimulate  $\beta\beta$  TCR T cells are generally the types of antigens that are taken up by dendritic cells in Peyer's patches that lead to Th2 T cell differentiation. Another, non-mutually exclusive, possibility is that  $\beta\beta$  TCR T cells manifesting a Th2 phenotype have better survivability than their Th1 counterparts, again because of the cytokine milieu in which they develop. This possibility derives from the observation that stimulation of T cells from TCR- $\alpha$  chain-deficient mice with epithelial cells both under Th1 and Th2 conditions leads to poorer survival in the former instance than in the latter (33). Moreover, the surviving Th2 T cells display evidence of oligoclonality and can transfer disease, whereas the Th1 T cells do not. These data suggest not only that antigens stimulating  $\beta\beta$  TCR T cells only do so under a Th2 condition but also that such stimulation under a Th1 condition leads to a different pattern of clonal stimulation and T cells that are subject to apoptosis.

A final point to emerge from the study of TCR- $\alpha$  chain-deficient mice relates to the role of B cells in the pathogenesis of this model of inflammation and, by extension, in ulcerative colitis. In particular, it was found that double mutant TCR- $\alpha$  chain-deficient  $\mu$ Ig-deficient mice somewhat paradoxically develop more severe colitis than single mutant TCR- $\alpha$  chain-deficient mice (63). Furthermore, transfer of mesenteric lymph nodes (MLN) cells from the double mutant to Rag-2-deficient mice produced colitis in the latter, which was abolished by the cotransfer of B cells and the coadministration of purified Ig or monoclonal antibodies reactive with colonic epithelial cells from the TCR- $\alpha$  chain-deficient mice. This decreased disease with B cells or B cell products (autoantibodies) was associated with decreased numbers of apoptotic cells in the epithelium and lamina propria and was attributed to decreased clearance of these cells mediated by the autoantibodies (63). Another explanation, however, is that the B cells produce regulatory cytokines that suppress disease (and also affect apoptosis). Indeed, preliminary studies suggest that B cells express high levels of CD1d and secrete IL-10 (A. Mizoguchi, R.J. Blumberg & A. Bhan, personal communication). In any case, these studies show that B cells or the autoantibodies they produce do not play a pathogenic role in TCR- $\alpha$  chain-deficient mice and may actually ameliorate disease. In addition, this suggests that autoantibodies in ulcerative colitis are likewise nonpathogenic.

## Oxazalone Colitis

Whereas administration of TNBS to SJL/J mice leads to colitis driven by polarized Th1 T cell responses, administration of another haptening agent, oxazalone, leads to a colitis caused by a polarized Th2 T cell response. Oxazalone colitis, however, is a considerably different disease than its TNBS counterpart (31). First, when administered intrarectally without prior sensitization, it develops more quickly and resolves more quickly than TNBS colitis, usually within 4–5 days; in addition, it

produces a more superficial inflammation that affects the distal half of the colon rather than the whole colon. Finally, rather than producing an intense infiltrative inflammation that obliterates villous architecture, it produces inflammation that generally maintains villous architecture but is associated with bowel wall edema and luminal exudates. Overall, the lesion is more reminiscent of ulcerative colitis than Crohn's disease, but the examination of a more chronic oxazolone colitis would be necessary to verify this view. If mice are presensitized with subcutaneous oxazolone, a more chronic lesion ensues that lasts on the order of 1–2 weeks; this lesion retains the characteristics described above for the more acute lesion and lends credence to the idea that oxazolone colitis is indeed an ulcerative-colitis-like inflammation (F. Scheiffele, I. Fuss, W. Strober, unpublished observations).

The cytokine response of oxazolone colitis is also very different from that in TNBS colitis (31). It is dominated by a high IL-4 and IL-5 response, but a normal or reduced IFN- $\gamma$  response. This Th2 response is in fact the cause of the inflammation, as shown by the fact that anti-IL-4 administration abolishes disease, whereas an anti-IL-12 administration exacerbates the disease and causes a pancolitis. Another notable feature of the cytokine response in oxazolone colitis is a marked TGF- $\beta$  response that is higher in the proximal colon than in the distal colon. In fact, the high TGF- $\beta$  response may be responsible for the short duration of disease as well as its limitation to the distal colon. This is suggested by the fact that, as mentioned above, TGF- $\beta$  production is higher in the proximal colon, as well as the fact that anti-TGF- $\beta$  treatment leads to severe pancolitis.

The reason lamina propria cells in SJL/J mice respond to oxazolone with a Th2 response rather than a Th1 response is unclear. One cannot invoke the idea that the presence of T cells with abnormal TCRs that only respond to antigens that induce Th2 differentiation because there is nothing to show that the T cell profiles of the responding mice are abnormal. A more likely possibility arises from emerging evidence that during the induction of oxazolone colitis, oxazolone is presented to T cells by APCs in the context of an atypical MHC class I molecule, CD1d, and that the interacting T cell is an NK T cell that is the effector cell causing the colitis (F. Scheiffele, I. Fuss, W. Strober, unpublished observations). Thus, one might postulate that this somewhat unique interaction preferentially results in Th2 T cell differentiation. Some evidence in support of this possibility is inherent in older studies showing that NK T cells have a propensity to produce IL-4, as well as newer studies showing that mice with a targeted deletion of the chemokine receptor, CCR5, when challenged with dextran sulfate sodium (DSS) to produce DSS colitis develop lesions containing T cells producing IL-4 (179). Whereas the reason mice with this deletion manifest this kind of response is not really known, one might postulate that the absence of CCR5 leads to decreased Th1 responses and thus the preferential expansion of NK T cells that inherently produces Th2 cytokines. It should be noted that in CCR5-deficient mice, DSS colitis is less severe than in normal mice, indicating that the NK T cells developing in this situation appear to be regulatory cells. This is in contrast to the situation in oxazolone colitis where, as mentioned, the NK T cells are effector cells. Whether NK T cells act as

regulatory cells in the context of DSS colitis because they produce IL-4 or because of other factors remains to be determined.

Aside from the nature and origin of the effector cell causing disease in oxazalone colitis is the question of the downstream inflammatory cytokines causing disease. One possibility that requires further study is that oxazalone colitis leads to production of IL-4, which in turn leads to the secretion of other cytokines, such as IL-9 and IL-13.

## MODELS OF COLITIS RELATED TO DEFECTS IN EPITHELIAL CELL BARRIER FUNCTION

A number of diverse models of colitis have been discovered that are due to defects in epithelial cell barrier function. It should be emphasized, however, that such defects in the present context are broadly defined to include both barrier function relating to permeability to macromolecules and barrier function involving processes that enable the intestinal epithelial cell to secrete immune mediators. The latter type of defect could take the form of inadequate secretion of mediators that thereby increases the exposure of the mucosal system to antigens in the mucosal microflora or to excessive secretion of mediators and the initiation of inflammation by the stimulation of “professional” cellular secretors of inflammatory cytokines (macrophages). In addition to the models described below, two models already discussed (colitis associated with IL-2 deficiency and with IL-10 deficiency) have been shown to have abnormal epithelial cell barrier function. In these cases it is likely that the latter is secondary to a more primary abnormality as it is known that both Th1 and Th2 cytokines can influence barrier function in various ways.

### Colitis Associated with Dominant-Negative N-Cadherin Expression

Cadherins are transmembrane glycoproteins that mediate adherence between many cell types including intestinal epithelial cells. On the cytoplasmic side they bind to the cytoskeleton via interactions with  $\beta$ -catenin and on the cell surface, and they enter homophilic interactions with cadherins on neighboring cells (180). Recently, Hermiston & Gordon created a model with disrupted epithelial cell cadherin function by expressing a dominant-negative N-cadherin in epithelial cells that interfere with normal expression of E-cadherin (64). In particular, they inserted embryonic stem cells with an N-cadherin gene lacking an extracellular domain under the control of a small intestinal epithelial cell promoter (the fatty acid binding protein promoter) into blastocysts to obtain chimeric mice that displayed patches of epithelial cells with poor cell-cell adhesion. The chimeric mice developed transmural cellular infiltration, cell crypt abscesses, goblet cell depletion, and both aphthous and linear ulcers in lamina propria areas subjacent to the epithelial patches containing

cells with defective adherence but not in areas subjacent to epithelial patches with normally adherent epithelial cells.

The most reasonable explanation for this pattern of inflammation is that in areas of the mucosa where there is breakdown of the epithelial barrier (owing in this case to defective cell-cell adhesion) there is excessive exposure of mucosal lymphoid elements (normal mucosal microflora), which subsequently leads to a nonhomeostatic immune response and mucosal inflammation. This picture is thus not unlike that in TNBS colitis, in which the introduction of TNBS in the presence of the substance (ethanol) that disrupts the mucosal barrier leads to an unbalanced immune response and subsequent inflammation. As to the question of why such exposure leads to inflammation, it can be postulated that any exposure of the mucosal immune system to antigens in a manner that bypasses the Peyer's patches leads to an inadequate regulatory T cell response because such cells preferentially develop in the organized lymphoid tissue of the mucosa. Finally, because the N-cadherin dominant-negative model of inflammation occurs in the vicinity of porous epithelial cells despite the fact that the microbial microflora are identical in both nonporous and porous areas, it is an exquisite demonstration of the fact that antigens in normal mucosal microflora are sufficient for the induction of responses that lead to disease.

### Colitis in *mdr1a*-Deficient Mice

A second and equally interesting model of colitis related to barrier function is a mouse model characterized by deficiency in the *mdr1a* gene (65). This gene is one of several "multiple drug-resistant" (*mdr*) genes expressed in many cells (including epithelial cells) that belong to a family of transporter proteins that pump small amphiphilic and hydrophobic molecules out of the cell and thus confer drug resistance (181, 182). This model was created because the gene encoding the *mdr1a* transporter is present in a region of the human genome that is thought to harbor a disease gene that leads to inflammatory bowel disease (183, 184). Bone marrow transfer studies involving wild-type donor cells into *mdr1a*-deficient recipients demonstrated that the colitis develops in *mdr1a*-deficient mice because of the deficiency of *mdr1a* in epithelial cells rather than in lymphoid or myeloid cells (65). Thus, the model allows one to focus on the role of epithelial cells in mucosal inflammation.

Colitis developing in *mdr1a*-deficient mice is a spontaneous colitis consisting of a transmural T cell and B cell infiltration that is similar to that found in Crohn's disease (despite the fact that it has been called a model of ulcerative colitis). It is important to mention, however, that the epithelial cells in *mdr1a*-deficient mice are arrayed in long, dysregulated crypts that are associated with crypt abscesses and surface ulcerations (65). The Crohn's disease-like picture was borne out by the presence of an mRNA cytokine profile indicative of a Th1-mediated inflammation (J. Viney, personal communication).

Recently, it has been shown that mucosal organisms can profoundly influence epithelial cell function with respect to cytokine/chemokine secretion. This influence can be negative in that epithelial cell interactions with certain nonpathogenic

organisms leads to a block in ubiquitination of  $I\kappa B\alpha$  (necessary for  $I\kappa B\alpha$  degradation) and thus a subsequent block in NF- $\kappa$ B translocation to the nucleus (185). This influence can also be positive because bacterial flagellin, signaling through TLR-5, leads to NF- $\kappa$ B expression (186). If these events are influenced or caused by bacterial products entering the cell, we can see how epithelial cell function can be negatively or positively impacted by a defect in a transporter mechanism. Furthermore, we can understand how such negative or positive perturbation could lead to increased epithelial cell production of chemokines and cytokines that lead to the influx of inflammatory elements into the epithelial layer. Thus, it seems possible that the *mdr1a*-deficient mouse does not develop inflammation because of increased epithelial layer permeability per se but because of increased bacterially induced activation of epithelial cells.

## Dextran Sulfate Colitis

Yet another model of colitis that is at least partially related to a change in epithelial cell barrier function is the colitis induced by the physical agent, dextran sulfate sodium. This is a relatively old model that has been frequently used to study the efficacy of potential therapeutic agents because of its ease to induce via administration of DSS in drinking water and because DSS induces a consistent level of colitis with a defined onset (18–22). As mentioned in the Introduction, DSS colitis can be induced in Rag-2-deficient or thymectomized mice (22). This argues that the mechanisms of inflammation in this form of colitis are, at least initially, the activation of nonlymphoid cells such as macrophages and the release of pro-inflammatory cytokines (187, 188). Changes in epithelial barrier function as measured by permeability of the intestinal wall to Evan's blue can be found early (several days before the onset of frank inflammation) (19) and thus may set the stage for macrophage activation. The relation of DSS colitis to epithelial barrier function is further suggested by the fact that administration of DSS to mice with deficiency of intestinal trefoil factor, a factor important to maintenance and repair of the epithelial layer, leads to a far more severe colitis than observed in normal mice (189). However, this may be a result of the fact that epithelial cell layer integrity plays a role in the initiation of DSS colitis as indicated above, or because reestablishment of such integrity is a condition of colitis resolution. In the acute stages of DSS colitis the (secondary?) T cell response consists of a polarized Th1 response, but in later and more chronic phases of the inflammation, a mixed Th1/Th2 response occurs (188). In either case, DSS elicits the secretion of large amounts of TNF- $\alpha$  and IL-6, which are mainly responsible for the tissue damage in the disease.

Whereas antigens in the mucosal microflora probably play a role in the production of DSS colitis, it has recently been shown that they also play a role in the suppression (and resolution) of the colitis. This is shown by the fact that mice administered  $\alpha$ -galactosylceramide (a glycolipid antigen that activates NK T cells when presented to them in the context of CD1d, a nonclassical MHC class I antigen-presenting molecule expressed on epithelial cells and other APCs)

manifest decreased DSS colitis as compared with mice administered a control glycolipid without these properties. This improvement is not seen in mice deficient in CD1d or in Rag<sup>-/-</sup> negative mice deficient in T cells (and NK T cells) (61). Finally, by confocal microscopy the administered  $\alpha$ -galactocylceramide could be localized to the epithelium. These studies reveal that NK T cells stimulated by glycolipid antigen play a protective role in DSS colitis that is not unlike their role in the SCID colitis model mentioned above. The mechanism of this role is not yet defined, but given the localization of the stimulating antigen to the epithelium, the NK T cells may be secreting substances that reestablish epithelial integrity or regulate other aspects of the mucosal environment that normally drive the colitis in this model. As discussed in relation to NK T cells found in CCR5-deficient mice, this may involve the secretion of Th2 cytokines.

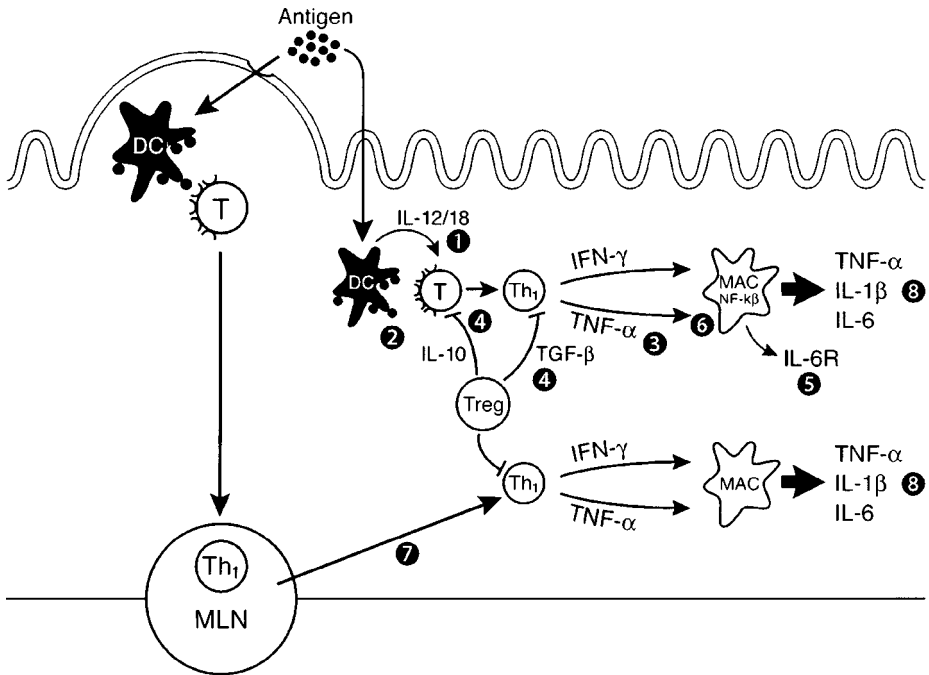
## TREATMENT OF MODELS OF MUCOSAL INFLAMMATION

The fact that models of mucosal inflammation, whatever their underlying cause, resolve themselves into either Th1 or Th2 T cell-mediated inflammation has led to the recognition that models of vastly different etiologies can be treated with agents that block these final common pathways at any of a variety of points. As shown in Figure 2, “points of attack” in the Th1 pathway can readily be identified and can be used to block the pathway in both models of inflammation and human Crohn’s disease. A similar diagram can be drawn with respect to the Th2 pathway, which can be applied to Th2 models of inflammation and perhaps human ulcerative colitis.

Only the broad outline of such treatment approaches can be discussed in this review. With respect to Th1-mediated inflammation, the use of agents that block IL-12 secretion or IL-12 activity provides the most direct approach because, as we have seen, depriving Th1 cells of IL-12 leads to their apoptosis (32, 123). It should be noted that not only anti-IL-12, but also other agents that downregulate IL-12 secretion are possible therapeutic agents in this context (190, 191). A related kind of therapy involves the use of anti-TNF- $\alpha$  antibody and soluble TNF-R agents that, as discussed above, block the Th1 response at both the inductive and the effector phases of the response. This approach has already proven useful in the treatment of human Crohn’s disease (192).

A parallel approach to Th2-mediated inflammation is more problematic in that although anti-IL-4 may be an effective treatment of Th2 models, it does not apply to human ulcerative colitis because this disease has not been shown to be caused by IL-4 dysregulation. A promising alternative approach is to target IL-6R with the use of an anti-IL-6 receptor antibody (193, 194). Such therapy blocks IL-6 “transsignaling” and leads to the apoptosis of both Th1 and Th2 T cells. Thus, it is theoretically applicable to both Th1- and Th2-mediated inflammation.

It is also possible that models of inflammation can be treated with regulatory (suppressive) cytokines such as IL-10 or TGF- $\beta$ . In studies conducted so far IL-10 has been applied with mixed success and, likewise, has been only marginally effective in human IBD (195, 196). The problem may be one of cytokine localization



**Figure 2** As shown in this diagram of Th1 T cell–mediated mucosal inflammation, the Th1 pathway can be “attacked” (i.e., inhibited or disrupted) at many different points, each representing a potential means of therapeutic intervention. These points are defined as follows: 1) inhibitors of IL-12/IL-18 (i.e., anti-IL-12, rCT-B, or  $\beta$ -agonists); 2) inhibitors of DC-T cell interaction (i.e., anti-CD40L or anti-CD134); 3) inhibitors of TNF- $\alpha$  (i.e., anti-TNF- $\alpha$  and TNF- $\alpha$ R); 4) IL-10 and TGF- $\beta$  (i.e., TGF- $\beta$  or IL-10 plasmids or administration of Th3 or Tr1 cells); 5) inhibition of IL-6 *trans*-signaling, anti-IL-6R; 6) NF- $\kappa$ B inhibitors; 7) inhibitors of homing or adhesion (i.e., anti- $\alpha$ 4 $\beta$ 7, anti- $\alpha$ E $\beta$ 7, anti-CD44v7, or anti-sense oligos to ICAM1); 8) downstream inhibitors of TNF- $\alpha$  (i.e., phosphodiesterase inhibitor 4, pentoxifylline, thalidomide, or metalloproteinase inhibitors).

because there is one report that IL-10 delivered by a *Lactococcus lactis* organisms was effective in two forms of experimental colitis (197). The use of TGF- $\beta$  has been explored in studies in which plasmids encoding TGF- $\beta$  are administered intranasally (29). As mentioned above, this leads to cells producing TGF- $\beta$  that migrate to mucosal tissues that are capable of reversing established TNBS colitis. A possible objection to this approach is that TGF- $\beta$  can induce fibrosis; however, the plasmid also induces IL-10 secretion, which appears to suppress TGF- $\beta$ -induced fibrosis (198).

Other approaches to the treatment of models of mucosal inflammation include the use of agents that target homing and localization of inflammatory cells. This

includes antibodies to integrins (anti-MadCAM-1, anti- $\alpha_E\beta_7$  and anti-CD44v7 as well as anti-sense oligonucleotides that interfere with integrin synthesis (199–201). Finally, attempts to control the mucosal inflammation by the use of agents that block the NF- $\kappa$ B pathway have been tested with some success in murine models (202–203). The question is whether such therapy will cause unacceptable toxicity when applied to humans.

## CLOSING CONSIDERATIONS

In this review of models of mucosal inflammation we have sought to emphasize recurrent characteristics of the models that allow them to be understood within a more or less consistent framework. This is perhaps best encapsulated by the fact that the models are invariably associated with one or another genetically determined or induced immune imbalance that ultimately expresses itself as a type 1 defect owing to excessive effector cell response or a type 2 defect owing to an inadequate regulatory cell response. Furthermore, in both the type 1 and type 2 defects, the response takes the form of either a Th1 or Th2 T cell-mediated inflammation that is driven not by antigens associated with exogenous pathogenic organisms but by antigens associated with the normal mucosal microflora. Given the fact that such antigens are the equivalent of self-antigens, the models may thus be visualized as a special type of autoimmunity that takes on a somewhat unique form because it involves effector and regulatory cell mechanisms that are characteristic of mucosal responses.

The impact of the knowledge gained from the study of models of inflammation on the understanding of human IBDs is difficult to exaggerate. Thus, it is fair to say that the framework used to visualize the pathogenesis of these diseases is currently derived largely from the murine models and, in turn, new patient-oriented research is mainly motivated by one or another aspect of the models. This includes research on new treatments of the disease that are either suggested by the models or are tested in the models. Looking ahead to the emerging area of genetic research in IBDs, the models will be an essential tool in the identification of genes that determine susceptibility and resistance to these diseases and thus the genes that will enable their genetic manipulation.

**Visit the Annual Reviews home page at [www.annualreviews.org](http://www.annualreviews.org)**

## LITERATURE CITED

1. Strober W. 1985. Animal models of inflammatory bowel disease—an overview. *Dig. Dis. Sci.* 30:(Suppl.):3–8
2. Kim HS, Berstad A. 1992. Experimental colitis in animal models. *Scand. J. Gastroenterol.* 27:529–37
3. Kirsner JB. 1961. Experimental “colitis” with particular reference to hypersensitivity reactions in the colon. *Gastroenterology* 40:307–12
4. Kraft SC, Fitch FW, Kirsner JB. 1963. Histologic and immunohistochemical

- features of Auer "colitis" in rabbits. *Am. J. Pathol.* 43:913–23
5. Mee AS, McLaughlin JE, Hodgson HJ, Jewell DP. 1979. Chronic immune colitis in rabbits. *Gut* 20:1–5
  6. Halpern B, Zweibaum A, Oriol Palou R, Morard JC. 1967. Experimental immune ulcerative colitis. In *Immunopathology, International Symposium*, pp. 161–78. Basel/Stuttgart: Schwabe and Co.
  7. Rabin BS, Rogers SJ. 1978. A cell-mediated immune model of inflammatory bowel disease in the rabbit. *Gastroenterology* 75:29–33
  8. Glick ME, Falchuk ZM. 1981. Dinitrochlorobenzene-induced colitis in the guinea-pig: studies of colonic lamina propria lymphocytes. *Gut* 22:120–25
  9. MacPherson BR, Pfeiffer CJ. 1978. Experimental production of diffuse colitis in rats. *Digestion* 17:135–50
  10. Sharon P, Stenson WF. 1985. Metabolism of arachidonic acid in acetic acid colitis in rats: similarity to human inflammatory bowel disease. *Gastroenterology* 88:55–63
  11. Fretland DJ, Widomski DL, Levin S, Gaginella TS. 1990. Colonic inflammation in the rabbit-induced by phorbol-12-myristate-13-acetate. *Inflammation* 14:143–50
  12. Marasco WA, Phan SH, Krutzch H, et al. 1984. Purification and identification of formyl-methionyl-leucyl-phenylalanine as the major peptide neutrophil chemotactic factor produced by *Escherichia coli*. *J. Biol. Chem.* 259:5430–39
  13. Williams LT, Synderman R, Pike MC, Lefkowitz RJ. 1977. Specific receptor site for chemotactic peptides on human polymorphonuclear leukocytes. *Proc. Natl. Acad. Sci. USA* 74:1204–8
  14. Painter GR, Sklar LA, Jesatis AJ, Schmitt M, Cochrane CG. 1984. Activation of neutrophils by N-formyl chemotactic peptides. *Fed. Proc.* 43:2737–42
  15. Marcus R, Watt J. 1969. Seaweeds and ulcerative colitis in laboratory animals. *Lancet* 2:489–90
  16. Abraham R, Fabian RJ, Goldberg L, Coulston F. 1974. Role of lysosomes in carageenan-induced cecal ulceration. *Gastroenterology* :1169–81
  17. Watt J, Marcus R. 1972. Ulceration of the colon in rabbits fed sulfated amylopectin. *J. Pharm. Pharmacol.* 24:68–69
  18. Ohkusa T. 1985. Production of experimental ulcerative colitis in hamsters by dextran sulfate sodium and change in intestinal microflora. *Jpn. J. Gastroenterol.* 82:1327–36
  19. Kitajima S, Takoma S, Morimoto M. 1999. Changes in colonic mucosal permeability mouse colitis induced with dextran sulfate sodium. *Exp. Animal* 48:137–43
  20. Onderdonk AB, Hermos JA, Dzink JL, Bartlett JG. 1978. Protective effect of metronidazole in experimental ulcerative colitis. *Gastroenterology* 74:521–26
  21. Rath HC, Schultz M, Freitag R, et al. 2001. Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. *Infect. Immun.* 69:2277–85
  22. Axelsson LG, Landstrom E, Goldschmitt TJ, Gronberg A, Bylund-Fellenius AC. 1996. Dextran sulfate sodium (DSS) induced experimental colitis in immunodeficient mice: effects in CD4+ cell depleted, athymic and NK-cell depleted SCID mice. *Inflamm. Res.* 45:181–91
  23. Strober W, Fuss IJ, Ehrhardt RO, Neurath M, Boirivant M, Ludviksson BR. 1998. Mucosal immunoregulation and inflammatory bowel disease: new insights from murine models of inflammation. *Scand. J. Immunol.* 48:453–58
  24. Strober W, Kelsall B, Marth T. 1998. Oral tolerance. *J. Clin. Immunol.* 18:1–30
  25. Ludviksson BR, Seegers D, Resnick AS, Strober W. 2000. The effect of TGF-beta1 on immune responses of naïve versus memory CD4+ Th1/Th2 T cells. *Eur. J. Immunol.* 30:2101–11
  26. Seder RA, Marth T, Sieve MC, Letterio

- JJ, Roberts AB, Kelsall B. 1998. Factors involved in the differentiation of TGF-beta-producing cells from naïve CD4+ T cells: IL-4 and IFN- $\gamma$  have opposing effects, while TGF-beta positively regulates its own production. *J. Immunol.* 160:5719–28
27. Marth T, Strober W, Seder RA, Kelsall BL. 1997. Regulation of transforming growth factor-beta production by interleukin-12. *Eur. J. Immunol.* 27:1213–20
28. Pardoux C, Ma X, Gobert S, Pellegrini S, Mayeux P, Gay F, Trinchieri G, Chouaib S. 1999. Downregulation of interleukin-12 (IL-12) responsiveness in human T cells by transforming growth factor-beta: relationship with IL-12 signaling. *Blood* 93:1448–55
29. Kitani A, Fuss IJ, Nakamura K, Schwartz OM, Usui T, Strober W. 2000. Treatment of experimental (trinitrobenzene sulfonic acid) colitis by intranasal administration of transforming growth factor (TGF)-beta1 plasmid: TGF-beta1-mediated suppression of T helper cell type 1 response occurs by interleukin (IL)-10 induction and IL-12 receptor beta2 chain downregulation. *J. Exp. Med.* 192:41–52
30. Xu H, Zhang GX, Wysocka M, Wu CY, Trinchieri G, Rostami A. 2000. The suppressive effect of TGF-beta on IL-12-mediated immune modulation specific to a peptide Ac1-11 of myelin basic protein (MBP): a mechanism involved in inhibition of both IL-12 receptor beta1 and beta2. *J. Neuroimmunol.* 108:53–63
31. Boirivant M, Fuss IJ, Chu A, Strober W. 1998. Oxazolone colitis: a murine model of T helper cell type 2 colitis treatable with antibodies to interleukin 4. *J. Exp. Med.* 188:1929–39
32. Neurath MF, Fuss I, Kelsall BL, Stüber E, Strober W. 1995. Antibodies to interleukin 12 abrogate established experimental colitis in mice. *J. Exp. Med.* 182:1281–90
33. Mizoguchi A, Mizoguchi E, Saubermann LJ, Higaki K, Blumberg RS, Bhan AK. 2000. Limited CD4 T-cell diversity associated with colitis in T-cell receptor alpha mutant mice requires a T helper 2 environment. *Gastroenterology* 119:983–95
34. Takahashi I, Iijima H, Katashima R, Itakura M, Kiyono H. 1999. Clonal expansion of CD4+ TCRbeta beta+ T cells in TCR alpha-chain-deficient mice by gut-derived antigens. *J. Immunol.* 162:1843–50
35. Spencer DM, Banerjee S, Veldmann GM, Levine AD. 1999. Anti-IL-12 therapy in IL-10 deficient mice prevents the onset of colitis and reverses early diseases, but is ineffective in late disease. *Gastroenterology* 116:G3298 (Abstract)
36. Kontoyiannis D, Pasparakis M, Pizarro T, Cominelli F, Kollias G. 1999. Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity* 10:387–98
37. Matsumoto S, et al. 1998. Inflammatory bowel disease-like enteritis and caecitis in a senescence accelerated mouse P1/Yit strain. *Gut* 43:71–78
38. Kosiewicz MM, Nast CC, Krishnan A, Rivera-Nieves J, Moskaluk CA, Matsumoto S, Kozaiwa K, Cominelli F. 2001. Th1-type responses mediate spontaneous ileitis in a novel murine model of Crohn's disease. *J. Clin. Invest.* 107:695–702
39. Fuss IJ, Neurath MF, Boirivant M, Klein JS, de la Motte C, Strong SA, Fiocchi C, Strober W. 1996. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN- $\gamma$ , whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J. Immunol.* 157:1261–70
40. Mullin GE, Maycon ZR, Braun-Elwert L, Cerchia R, James SP, Katz S, Weissman GW, McKinley MJ, Fisher SE. 1996. Inflammatory bowel disease mucosal biopsies have specialized lymphokine mRNA profiles. *Inflamm. Bowel Dis.* 2:16–26

41. Monteleone G, Biancone L, Marasco R, Marrone G, Marasco O, Luzzaf F, Pallone F. 1997. Interleukin-12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology* 112:1169-78
42. Parronchi P, Romagnì P, Annunziato F, Sampognaro S, Becchio A, Giannarini L, Maggi E, Pupilli C, Tonelli F, Romagnì S. 1997. Type 1 T-helper cell predominance and IL-12 expression in the gut of patients with Crohn's disease. *Am. J. Pathol.* 150:823-32
43. Malmstrom V, Shipton D, Singh B, Al-Shamkhani A, Puklavec MJ, Barclay AN, Powrie F. 2001. CD134L expression on dendritic cells in the mesenteric lymph nodes drive colitis in T cell-restored SCID mice. *J. Immunol.* 166:6972-81
44. Takeda K, Clausen BE, Kaisho T, Tsujimura T, Terada N, Forster I, Akira S. 1999. Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of STAT3 in macrophages and neutrophils. *Immunity* 10:39-49
45. Okamoto S, Watanabe M, Yamazaki M, Yajima T, Hayashi T, Ishii H, Mukai M, Yamada T, Noriaki W, Jameson BA, Hibi T. 1999. A synthetic mimetic of CD4 is able to suppress disease in rodent model of immune cells. *Eur. J. Immunol.* 29:355-66
46. Simpson SJ, Mizoguchi E, Allen D, Bhan AK, Terhorst C. 1995. Evidence that CD4+ but not CD8+ T cells are responsible for murine interleukin-2-deficient colitis. *Eur. J. Immunol.* 25:2618-25
47. Simpson SJ, de Jon YP, Shah SA, Comiskey M, Wang B, Spielman JA, Podack ER, Mizoguchi E, Bhan AK, Terhorst C. 1998. Consequences of Fas-ligand and perforin expression by colon T cells in a mouse model of inflammatory bowel disease. *Gastroenterology* 115:849-55
48. Strater J, Wellisch I, Riedl S, Walczak H, Koretz K, Tandara A, Krammer PH, Moller P. 1997. CD95 (APO-1/Fas)-mediated apoptosis in colon epithelial cells: a possible role in ulcerative colitis. *Gastroenterology* 113:160-67
49. Mizoguchi E, Mizoguchi A, Bhan AK. 1997. Role of cytokines in the early stages of chronic colitis in TCR $\alpha$ -mutant mice. *Lab. Invest.* 76:385-97
50. Miyashita-Kawaguchi M, Shin-ichiro S, Korosu H, Kato-Nagoka N, Matsuoko Y, Ohwukim M, et al. 2001. An accessory role of TCR  $\gamma\delta$  cells in the exacerbation of inflammatory bowel disease in TCR $\alpha$  mutant mice. *Eur. J. Immunol.* 31:980-88
51. Mombaerts P, Mizoguchi E, Grusby MJ, Glimcher L, Bhan AK, Tonegawa S. 1993. Spontaneous development of inflammatory bowel disease in T cell receptor mutant mice. *Cell* 75:275-82
52. Hoffmann JC, Peters K, Henschke S, Hermann B, Pfister K, Westermann J, Zeitz M. 2001. Role of T lymphocytes in rat 2, 4, 6-trinitrobenzene sulphonic acid induced colitis: increased mortality after  $\gamma\delta$  T cell depletion and no effect of  $\alpha\beta$  T cell depletion. *Gut* 48:489-95
53. Boismenu R, Havren WL. 1994. Modulation of epithelial cell growth by intraepithelial  $\gamma\delta$  T cells. *Science* 266:1253-55
54. Seder RA, Marth T, Sieve MC, Strober W, Letterio JJ, Roberts AB, Kelsall B. 1998. Factors involved in the differentiation of TGF-beta-producing cells from naïve CD4+ T cells: IL-4 and IFN- $\gamma$  have opposing effects, while TGF-beta positively regulates its own production. *J. Immunol.* 160:5719-28
55. Groux H, O'Garra A, Bigler M, Rouleau M, Antonenko S, de Vries JE, Roncarolo MG. 1997. A CD4+ T-cell subset inhibits antigen-specific-T-cell responses and prevents colitis. *Nature* 389:737-42
56. Levings MK, Sangregorio R, Galbiati F, Squadrone S, de Waal Malefyt R, Roncarolo MG. 2001. IFN-alpha and IL-10 induce the differentiation of human type 1 T regulatory cells. *J. Immunol.* 166:5530-39
57. Shevach EM, Thornton AM. 1998. CD4+

- CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. *J. Exp. Med.* 188:287–96
58. Takashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S. 1998. Immunologic self-tolerance maintained by CD25+ CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol.* 10:1969–80
59. Nakamura K, Kitani A, Strober W. 2001. Cell contact-dependent immunosuppression by CD4+/CD25+ regulatory T cells is mediated by cell surface-bound transforming growth factor- $\beta$ . *J. Exp. Med.* 194:629–44
60. Fort MM, Leach MW, Rennick DM. 1998. A role for NK cells as regulators of CD4+ T cells in a transfer model of colitis. *J. Immunol.* 161:3256–61
61. Saubermann LJ, Beck P, De Jong YP, Pittmann RS, Ryan MS, Kim HS, et al. 2000. Activation of natural killer T cells by alpha-galactosylceramide in the presence of CD1d provides protection against colitis in mice. *Gastroenterology* 119:119–28
62. Blumberg RS, Gerdes D, Chott A, Porcellini SA, Balk SP. 1995. Structure and function of the CD1 family of MHC-like cell surface proteins. *Immunol. Rev.* 147:5–29
63. Mizoguchi A, Mizoguchi E, Smith RN, Preffer FI, Bhan AK. 1997. Suppressive role of B cells in chronic colitis of T cell receptor  $\alpha$  mice. *J. Exp. Med.* 10:1749–56
64. Hermiston ML, Gordon JI. 1995. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science* 270:1203–7
65. Panwala CM, Jones JC, Viney JL. 1998. A novel model of inflammatory bowel disease: mice deficient for the multiple drug resistance gene, *mdr1a*, spontaneously develop colitis. *J. Immunol.* 161:5733–44
66. Madsen KL, Malfair D, Gray D, Doyle JS, Jewell LD, Fedorak RN. 1999. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm. Bowel Dis.* 5:262–70
67. Mestecky J, Husby S, Moldoveanu Z, Waldo FB, van den Wall Bake AW, Elson CO. 1996. Induction of tolerance in humans: effectiveness of oral and nasal immunization routes. *Ann. NY Acad. Sci.* 778:194–201
68. Komagata Y, Weiner HL. 2000. Oral tolerance. *Rev. Immunogenet.* 2:61–73
69. Garside P, Mowat AM. 2001. Oral tolerance. *Semin. Immunol.* 13:177–85
70. Iwasaki A, Kelsall BL. 1999. Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J. Exp. Med.* 190:229–39
71. Iwasaki A, Kelsall BL. 2001. Unique functions of CD11b+, CD8 alpha+, and double-negative Peyer's patch dendritic cells. *J. Immunol.* 166:4884–90
72. Wirtz S, Finotto S, Lohse AW, Blessing M, Lehr HA, Galle PR, Neurath MF. 1999. Chronic intestinal inflammation in STAT-4 transgenic mice: characterization of disease and adoptive transfer by TNF-plus IFN- $\gamma$ -producing CD4+ T cells that respond to bacterial antigens. *J. Immunol.* 162:1884–88
73. Powrie F, Correa-Oliveira R, Mauze S, Coffman RL. 1994. Regulatory interactions between CD45RB<sup>high</sup> and CD45RB<sup>low</sup> CD4+ T cells are important for the balance between protective and pathogenic cell-mediated immunity. *J. Exp. Med.* 179:589–600
74. Lucas PJ, Kim SJ, Melby SJ, Gress RE. 2000. Disruption of T cell homeostasis in mice expressing a T cell-specific dominant negative transforming growth factor beta II receptor. *J. Exp. Med.* 191:1187–96
75. Gorelik L, Flavell RA. 2000. Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity* 12:171–81
76. Sartor RB. 1999. Microbial factors in

- the pathogenesis of Crohn's disease, ulcerative colitis, and experimental intestinal inflammation. In *Inflammatory Bowel Diseases*, ed. JB Kirsner, pp. 153–78. Philadelphia/London: Saunders. 5th ed.
77. Sartor RB, Veltkamp C. 2000. Interactions between enteric bacteria and the immune system which determine mucosal homeostasis vs. chronic intestinal inflammation: lessons from rodent models. In *IBD at the End of Its First Century*, ed. G Rogler, F Kullman, P Rutgeerts, RB Sartor, J Scholmerich, pp. 30–41. Dordrecht, The Netherlands: Kluwer Academic
78. Brimnes J, Reimann J, Nissen M, Claesson M. 2001. Enteric bacterial antigens activate CD4+ T cells from SCID mice with inflammatory bowel disease. *Eur. J. Immunol.* 31:23–31
79. Veltkamp C, Tonkonogy SL, De Jong YP, Albright C, Grenther WB, Balish E, Terhorst C, Sartor RB. 2001. Continuous stimulation by normal luminal bacteria is essential for the development and perpetuation of colitis in Tg(epsilon26) mice. *Gastroenterology* 120:900–13
80. Schultz M, Tonkonogy SL, Sellon RK, Veltkamp C, Godfrey VL, Kwon J, Grenther WB, Balish E, Horak I, Sartor RB. IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. *Am. J. Physiol. Gastrointest. Liver Physiol.* 276:G1461–72
81. Contractor NV, Bassirir H, Reya T, Park AY, Baumgart DC, Wasik MA, Emerson SG, Carding SR. 1998. Lymphoid hyperplasia, autoimmunity, and compromised intestinal intraepithelial lymphocyte development in colitis-free gnotobiotic IL-2-deficient mice. *J. Immunol.* 75:253–61
82. Bhan AK, Mizoguchi E, Smith RN, Mizoguchi A. 2000. Spontaneous chronic colitis in TCR alpha-mutant mice; an experimental model of human ulcerative colitis. *Int. Rev. Immunol.* 19:123–38
83. Axelsson LG, Midtvedt T, Bylund Fellenius AC. 1996. The role of intestinal bacteria, bacterial translocation and endotoxin in dextran sodium sulfate-induced colitis in the mouse. *Microb. Ecol. Health Dis.* 9:225–37
84. Tlaskalova H, Stepankova R, Hudcovic T, Kozakova H. 1999. The role of bacterial microflora in development of dextran sodium sulfate (DSS) induced colitis in immunocompetent and immunodeficient mice. *Microb. Ecol. Health Dis.* 11:115–16 (abstract)
85. Madsen KL, Doyle JS, Tavernini MM, et al. 2000. Antibiotic therapy attenuates colitis in interleukin-10 gene deficient mice. *Gastroenterology* 118:1094–105
86. Sadlack B, Merz H, Schorle H, Schimpl A, Feller AC, Horak I. 1993. Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. *Cell* 75:203–5
87. Duchmann R, Neurath MF, Meyer zum Buschenfelde KH. 1997. Responses to self and non-self intestinal microflora in health and inflammatory bowel disease. *Res. Immunol.* 148:589–94
88. Duchmann R, Schmitt E, Knolle P, Meyer zum Buschenfelde KH, Neurath M. 1996. Tolerance towards resident intestinal flora in mice is abrogated in experimental colitis and restored by treatment with interleukin-10 or antibodies to interleukin-12. *Eur. J. Immunol.* 26:934–38
89. Ehrhardt RO, Ludviksson BR, Gray B, Neurath M, Strober W. 1997. Induction and prevention of colonic inflammation in IL-2-deficient mice. *J. Immunol.* 158:566–73
90. Duchmann R, May E, Heike M, Knolle P, Neurath M, Meyer zum Buschenfelde, KH. T cell specificity and cross reactivity towards enterobacteria, *Bacterioides*, bifidobacterium, and antigens from resident intestinal flora in humans. *Gut* 44:812–18
91. Cong Y, Weaver CT, Lazenby A, Elson CO. 2000. Colitis induced by enteric bacterial antigen-specific CD4+ T cells require CD40-CD40 ligand interactions for

- a sustained increase in mucosal IL-12. *J. Immunol.* 165:2173–82
92. Cong Y, Brandwein SL, McCabe RP, Lazenby A, Birkenmeier EH, Sundberg JP, Elson CO. 1998. CD4+ T cells reactive to enteric bacterial antigens in spontaneously colitic C<sub>3</sub>H/HeJBir mice: increased T helper cell type 1 response and ability to transfer disease. *J. Exp. Med.* 16:855–64
  93. Elson C, Cong Y, Iqbal N, Weaver C. 2001. Immuno-bacterial homeostasis in the gut: new insights into an old enigma. *Semin. Immunol.* 13:187–94
  94. Aranda R, Sydora BC, McAllister PL, Binder SW, Yang HY, Targan SR, Kronenberg M. 1997. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4+, CD45RBhigh T cells to SCID recipients. *J. Immunol.* 158:3464–73
  95. Probert CS, Chott A, Turner JR, Saubermann LJ, Stevens AC, Bodinaku K, Elson CO, Balk SP, Blumberg RS. 1996. Persistent clonal expansion of peripheral blood CD4+ lymphocytes in chronic inflammatory bowel disease. *J. Immunol.* 157:3183–91
  96. Sellon RK, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. 1998. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect. Immun.* 66:5244–31
  97. Kishi D, Takahashi I, Kai Y, Tamagawa H, Iijima H, Obunai S, Nezu R, Ito T, Matsuda H, Kiyono H. 2000. Alteration of V beta usage and cytokine production of CD4+ TCR beta beta homodimer T cells by elimination of *Bacterioides vulgatus* prevents colitis in TCR alpha-chain-deficient mice. *J. Immunol.* 165:5891–99
  98. Rodloff AC, Widera P, Ehlers S, et al. 1990. Suppression of blastogenic transformation of lymphocytes by *Bacterioides fragilis* in vitro and in vivo. *Int. J. Med. Microbiol.* 274:406–16
  99. Kullberg MC, Ward JM, Gorelick PL, Caspar P, Hieny S, Cheever A, Jankovic D, Sher A. 1998. *Helicobacter hepaticus* triggers colitis in specific-pathogen-free interleukin-10 (IL-10)-deficient mice through an IL-12 and gamma interferon-dependent mechanism. *Infect. Immun.* 66:5157–66
  100. Dieleman LA, Arends A, Tonkonogy SL, Goerres MS, Craft DW, Grenther W, Sellon RK, Balish E, Sartor RB. *Helicobacter hepaticus* does not induce or potentiate colitis in interleukin-10-deficient mice. *Infect. Immunol.* 68:5107–13
  101. von Freeden-Jeffrey U, Davidson N, Wiler R, Fort M, Burdach S, Murray R. 1998. IL-7 deficiency prevents development of a non-T cell non-B cell-mediated colitis. *J. Immunol.* 161:5673–80
  102. Watanabe M, Ueno Y, Yajima T, Okamoto S, Hayashi T, Yamazaki M, Iwao Y, Ishii H, Haba S, Uehira M, Nishimoto H, Ishikawa H, Hata J, Hibi T. 1998. Interleukin 7 transgenic mice develop chronic colitis with decreased interleukin 7 protein accumulation in the colonic mucosa. *J. Exp. Med.* 187:389–402
  103. Rath HC, Wilson KH, Sartor RB. 1999. Differential induction of colitis and gastritis in HLA-B27 transgenic rats selectively colonized with *Bacterioides vulgatus* or *Escherichia coli*. *Infect. Immun.* 67:2969–74
  104. Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. 1999. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 116:1107–14
  105. Maggic-Price L, Shows D, Waggie K, Burich A, Zeng W, Morrissey P, Viney JL. 2001. Immunologic responses in Helicobacter-induced inflammatory bowel disease in multiple drug resistance (*mdr1a*-/-) mice. *Gastroenterology* 120: 2667 (abstract)
  106. Dalwadi H, Wei B, Kronenberg M,

- Sutton C, Braun J. 2001. The Crohn's disease-associated bacterial protein I2 is a novel enteric T cell superantigen. *Immunity* 15:149–58
107. Mizoguchi A, Mizoguchi E, Chiba C, Bhan AK. 1996. Role of appendix in the development of inflammatory bowel disease in TCR-alpha mutant mice. *J. Exp. Med.* 184:707–15
108. Mahler M, Bristol IJ, Leiter EH, Workman AE, Birkenmeier EH, Elson CO, Sundberg JP. 1998. Differential susceptibility of inbred mouse strains to dextran sulfate sodium-induced colitis. *Am. J. Physiol.* 274:6544–51
109. Stevceva L, Pavli P, Buffinton G, Wozniak A, Doe WF. 1999. Dextran sodium sulphate-induced colitis activity varies with mouse strain but develops in lipopolysaccharide-unresponsive mice. *J. Gastroenterol. Hepatol.* 14:54–60
110. Mahler M, Bristol IJ, Sundberg JP, Churchill GA, Birkenmeier EH, Elson CO, Leiter EH. 1999. Genetic analysis of susceptibility to dextran sulfate sodium-induced colitis in mice. *Genomics* 55:147–56
111. Maehler M, Most C, Schmidtke S, Hedrich HJ. 2001. Genetic analysis of susceptibility to colitis in IL-10 deficient mice. *Gastroenterology* 120:185 (Abstract)
112. Kozawa K, Sugawara K, Moskaluk CA, Matsumoto S, Cominelli F. 2001. Genetic analysis of susceptibility of ileitis in a spontaneous model of Crohn's disease. *Gastroenterology* 120:186 (Abstract)
113. Ahmad T, Satsangi J, McGovern D, Bunce M, Jewell DP. 2001. Review article: the genetics of inflammatory bowel disease. *Aliment. Pharmacol. Ther.* 18:1937–41
114. Ogura Y, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, et al. 2001. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 411:603–6
115. Hugot JP, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, et al. 2001. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 411:599–603
116. Beutler B. 2001. Autoimmunity and apoptosis: the Crohn's connection. *Immunity* 15:1–14
117. Morris GP, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. 1989. Hapten-induced model of colonic inflammation and ulceration in the rat colon. *Gastroenterology* 96:795–803
118. Yamada Y, Marshall S, Specian RD, Grisham MB. 1992. A comparative analysis of two models of colitis in rats. *Gastroenterology* 102:1524–34
119. Stüber E, Strober W, Neurath M. 1996. Blocking the CD40L-CD40 interaction in vivo specifically prevents the priming of T helper 1 cells through the inhibition of interleukin 12 secretion. *J. Exp. Med.* 183:693–98
120. Sadlack B, Lohler J, Schorle H, Klebb G, Haber H, SICKEL E, Noelle RJ, Horak I. 1995. Generalized autoimmune disease in interleukin-2-deficient mice is triggered by an uncontrolled activation and proliferation of CD4+ T cells. *Eur. J. Immunol.* 25:3055–59
121. De Jong YP, Comiskey M, Kalled SL, Mizoguchi E, Flavell RA, Bhan AK, Terhorst C. 2000. Chronic murine colitis is dependent on the CD154/CD40 pathway and can be attenuated by anti-CD154 administration. *Gastroenterology* 119:715–23
122. Neurath MF, Fuss I, Pasparakis M, Alexopoulou L, Haralambous S, Meyer zum Buschenfelde KH, Strober W, Kollias G. 1997. Predominant pathogenic role of tumor necrosis factor in experimental colitis in mice. *Eur. J. Immunol.* 27:1743–50
123. Fuss IJ, Marth T, Neurath M, Pearlstein GR, Jain A, Strober W. 1999. Anti-interleukin 12 treatment regulates apoptosis of Th1 T cells in experimental colitis in mice. *Gastroenterology* 117:1078–88

124. Neurath MF, Fuss IJ, Kelsall BL, Presky DH, Waegell W, Strober W. 1996. Experimental granulomatous colitis in mice is abrogated by induction of TGF- $\beta$ -mediated oral tolerance. *J. Exp. Med.* 183:2605–16
125. Elson CO, Beagley KW, Sharmanov AT, Fujihashi K, Kiyono H, Tennyson GS, Cong Y, Black CA, Ridwan BW, McGhee JR. 1996. Hapten-induced model of murine inflammatory bowel disease: mucosa immune response and protection by tolerance. *J. Immunol.* 157:2174–85
- 125a. Fuss IJ, Boirivant M, Lacey B, Strober W. 2002. The inter-related roles of TGF- $\beta$  and IL-10 in the regulation of experimental colitis. *J. Immunol.* In press
126. Powrie F, Leach MW, Mauze S, Caddle LB, Coffman RL. 1993. Phenotypically distinct subsets of CD4+ T cells induce or protect chronic intestinal inflammation in C.B-17 scid mice. *Int. Immunol.* 5:1461–71
127. Morrissey PJ, Charrier K, Braddy S, Liggitt D, Watson JD. 1993. CD4+ T cells that express high levels of CD45RB induce wasting disease when transferred into congenic severe combined immunodeficient mice. Disease development is prevented by cotransfer of purified CD4+ T cells. *J. Exp. Med.* 178:237–44
128. Powrie F, Leach MW, Mauze S, Menon S, Caddle LB, Coffman RL. 1994. Inhibition of Th1 responses prevents inflammatory bowel disease in scid mice reconstituted with CD45RBhi CD4+ T cells. *Immunity* 1:553–62
129. Leach MW, Bean AG, Mauze S, Coffman RL, Powrie F. 1996. Inflammatory bowel disease in C.B-17 scid mice reconstituted with the CD45RBhigh subset of CD4+ T cells. *Am. J. Pathol.* 148:1503–15
130. Claesson MH, Bregenholt S, Bonhagen K, Thoma S, Moller P, Grusby MJ, Leithauer F, Nissen MH, Reimann J. 1999. Colitis-inducing potency of CD4+ T cells in immunodeficient, adoptive hosts depends on their state of activation, IL-12 responsiveness, and CD45RB surface phenotype. *J. Immunol.* 162:3702–10
131. Matsuda JL, Gapin L, Sydora BC, Byrne F, Binder S, Kronenberg M, Aranda M. 2000. Systemic activation and antigen-driven oligoclonal expansion of T cells in a mouse model of colitis. *J. Immunol.* 164:2797–806
132. Powrie F, Carlino J, Leach MW, Mauze S, Coffman RL. 1996. A critical role for transforming growth factor- $\beta$  but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB<sup>low</sup> CD4+ T cells. *J. Exp. Med.* 183:2669–74
133. Read S, Malmstrom V, Powrie F. 2000. Cytotoxic T lymphocyte-associated antigen 4 plays an essential role in the function of CD25+CD4+ regulatory cells that control intestinal inflammation. *J. Exp. Med.* 192:295–302
134. Sakaguchi S, Toda M, Asano M, Itoh M, Morse SS, Sakaguchi N. 1996. T cell-mediated maintenance of natural self-tolerance: its breakdown as a possible cause of various autoimmune diseases. *J. Autoimmun.* 9:211–20
135. Bensingler SJ, Bandeira A, Jordon MS, Caton AJ, Laufer TM. 2001. Major histocompatibility complex class II-positive cortical epithelium mediates the selection of CD4+25+ immunoregulatory T cells. *J. Exp. Med.* 194:427–38
136. Hagenbaugh A, Sharma S, Dubinett SM, Wei SH, Aranda R, Cheroutre H, et al. 1997. Altered immune responses in interleukin-10 transgenic mice. *J. Exp. Med.* 185:2101–10
137. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. 1999. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J. Exp. Med.* 190:995–1004
138. Higgins LM, McDonald SA, Whittle N, Crockett N, Shields JG, MacDonald

- TT. 1999. Regulation of T cell activation in vitro and in vivo by targeting the OX40-OX40 ligand interaction: amelioration of ongoing inflammatory bowel disease with an OX40-IgG fusion protein, but not with an OX40 ligand-IgG fusion protein. *J. Immunol.* 162:486–93
139. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. 2000. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 100:665–69
140. Mullen AC, High FA, Hutchins AS, Lee HW, Villarino AV, Livingston DM, Kung AL, Cereb N, Yao TP, Yang SY, Reiner SL. 2001. Role of T-bet in commitment of Th1 cells before IL-12 dependent selection. *Science* 292:1907–10
141. Jacobson NG, Szabo J, Weber-Nordt RM, Zhong Z, Schreiber D, Darnell JE, Murphy KM. 1995. Interleukin 12 signaling in T helper type 1 (Th1) cells involves tyrosine phosphorylation of signal transducer and activator of transcription (STAT)3 and STAT4. *J. Exp. Med.* 181:1755–62
142. Kaplan MH, Sun YL, Hoey T, Grusby MJ. 1996. Impaired IL-12 responses and enhanced development of Th2 cells in STAT4-deficient mice. *Nature* :382–174
143. Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, Bradley A, Birnbaumer L. 1995. Gi2 alpha protein deficiency: a model of inflammatory bowel disease. *J. Clin. Immunol.* 15:1015–55
144. He J, Gurunathan S, Iwasaki A, Ash-Shaheed B, Kelsall BL. 2000. Primary role for Gi protein signaling in the regulation of interleukin 12 production and the induction of T helper cell type 1 responses. *J. Exp. Med.* 191:1605–10
145. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. 1993. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75:263–74
146. Kullberg MC, Rothfuchs AG, Jankovic D, Caspar P, Wynn TA, Gorelick PL, Cheever AW, Sher A. 2001. *Helicobacter hepaticus*-induced colitis in interleukin-10-deficient mice: cytokine requirements for the induction and maintenance of intestinal inflammation. *Infect. Immunol.* 69:4232–41
147. Fiorentino DF, Zlotnick A, Mosmann TR, Howard M, O'Garra A. 1991. IL-10 inhibits cytokine production by activated macrophages. *J. Immunol.* 147:3815–22
148. Trinchieri G. 1997. Cytokines acting on or secreted by macrophages during intracellular infection (IL-10, IL-12, IFN- $\gamma$ ). *Curr. Opin. Immunol.* 9:17–23
149. Ding L, Linsley PS, Huang LY, Germain RN, Shevach EM. 1993. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression. *J. Immunol.* 151:1224–34
150. Groux H, Bigler M, deVries JE, Roncarolo M. 1996. Interleukin-10 induces a long-term antigen-specific anergic state in human CD4+ T cells. *J. Exp. Med.* 184:19–29
151. Spencer SD, Di Marco F, Hooley J, Pitts-Meek S, Bauer M, Ryan AM, Sordat B, Gibbs VC, Aguet M. 1998. The orphan receptor CRF2-4 is an essential subunit of the interleukin 10 receptor. *J. Exp. Med.* 187:571–78
152. Suzuki A, Hanada T, Mitsuyama K, Takafumi Y, Kamizono S, Hoshino T, Kubo M, Yamashita A, Okabe M, Takeda K, Akira S, Matsumoto S, Toyonaga A, Sata M, Yoshimura A. 2001. CIS3/SOCS3/SSI3 plays a negative regulatory role in STAT3 activation and intestinal inflammation. *J. Exp. Med.* 193:471–81
153. Shull MM, Ormsby I, Kier AB, Pawloski S, Diebold RJ, Yin M, Allen R, Sidman C, Proetzel G, Calvin D, Annunziata N, Doetschman T. 1992. Targeted disruption of the mouse transforming growth factor- $\beta$ 1 gene results in multifocal inflammatory disease. *Nature* 359:693–99

154. Kulkarni AB, Huh C-G, Becker D, Geiser A, Lyght M, Flanders KC, Roberts AB, Sporn MB, Ward JM, Karlsson S. 1993. Transforming growth factor  $\beta$ 1 null mutation in mice causes excessive inflammatory response and early death. *Proc. Natl. Acad. Sci. USA* 90:770–74
155. Hahm KB, Im YH, Parks TW, Park SH, Markowitz S, Jung HY, Green J, Kim SJ. 2001. Loss of transforming growth factor beta signaling in the intestine contributes to tissue injury in inflammatory bowel disease. *Gut* 49:164–65
156. Simpson SJ, Shah S, Comiskey M, de Jong YP, Wang B, Mizoguchi E, Bhan AK, Terhorst C. 1998. T cell-mediated pathology in two models of experimental colitis depends predominantly on the interleukin 12/signal transducer and activator of transcription (STAT)-4 pathway, but is not conditional on interferon gamma expression by T cells. *J. Exp. Med.* 187:1225–34
157. Ludviksson BR, Ehrhardt RO, Strober W. 1997. TGF-beta production regulates the development of the 2,4,6-trinitrophenol-conjugated keyhole limpet hemocyanin-induced colonic inflammation in IL-2 deficient mice. *J. Immunol.* 159:3622–28
158. Hollander GA, Simpson SJ, Mizoguchi E, Nicogiannopoulou A, She J, Gutierrez-Ramos J, Bhan AK, Burakoff SJ, Wang B, Terhorst C. 1999. Severe colitis in mice with aberrant thymic selection. *Immunity* 3:27–38
159. Ludviksson BR, Gray B, Strober W, Ehrhardt RO. 1997. Dysregulated intrathymic development in the IL-2-deficient mouse leads to colitis-inducing thymocytes. *J. Immunol.* 158:104–11
160. Kneitz B, Herrmann T, Yonehara S, Schimpl A. 1995. Normal clonal expansion but impaired Fas-mediated cell death and anergy induction in interleukin-2-deficient mice. *Eur. J. Immunol.* 25:2572–77
161. Razi-Wolf Z, Hollander GA, Reiser H. 1996. Activation of CD4+ lymphocytes from interleukin-2-deficient mice by costimulatory B7 molecules. *Proc. Natl. Acad. Sci. USA* 93:2903–8
162. Telega GW, Baumgart DC, Carding SR. 2000. Uptake and presentation of antigen to T cells by primary colonic epithelial cells in normal and diseased states. *Gastroenterology* 119:1549–59
163. Willerford DM, Chen J, Ferry JA, Davidson L, Ma A, Alt FW. 1995. Interleukin-2 receptor  $\alpha$  chain regulates the size and content of the peripheral lymphoid compartment. *Immunity* 3:521–30
164. Poussier P, Ning T, Chen J, Banerjee D, Julius M. 2000. Intestinal inflammation observed in IL-2R/IL-2 mutant mice is associated with impaired intestinal T lymphopoiesis. *Gastroenterology* 118:880–91
165. Suzuki H, Kundig T, Furlonger C, Wakeham A, Timms E, Matsuyama T, Schmits R, Simard J, Ohashi PS, Grieser H, Taniguchi T, Paige C, Mak TW. 1995. Deregulated T cell activation and autoimmunity in mice lacking interleukin-2 receptor  $\beta$ . *Science* 268:1472–76
166. Wang B, Levelt C, Salio M, Zheng D, Sancho J, Liu CP, She J, Huang M, Higgins K, Sunshine MJ, et al. 1995. Over-expression of CD3 epsilon transgenes blocks T lymphocyte development. *Int. Immunol.* 7:435–58
167. Wang B, Simpson SJ, Hollander GA, Terhorst C. 1997. Development and function of T lymphocytes and natural killer cells after bone marrow transplantation of severely immunodeficient mice. *Immunol. Rev.* 157:53–60
168. Simpson SJ, Hollander GA, Mizoguchi E, Allen D, Bhan AK, Wang B, Terhorst C. 1997. Expression of pro-inflammatory cytokines by TCR $\alpha\beta$ + and TCR $\gamma\delta$ + T cells in an experimental model of colitis. *Eur. J. Immunol.* 27:17–25

169. Sundberg JP, Elson CO, Bedigian H, Birkenmeier EH. 1994. Spontaneous heritable colitis in a new substrain of C<sub>3</sub>H/HeJ mice. *Gastroenterology* 107:1726–35
170. Brandwein SL, McCabe RP, Cong Y, Waites KB, Ridwan BU, Dean PA, Ohkusa T, Birkenmeier EH, Sundberg JP, Elson CO. 1997. Spontaneously colitic C<sub>3</sub>H/HeJ Bir mice demonstrate selective antibody reactivity to antigens of the enteric bacterial flora. *J. Immunol.* 159:44–52
171. Sichler KJ, Overman KM, Sayed BA, Coroh B, Matsumoto S, Cominelli F, Pizarro TT. 2001. Dysregulated production of IEC-derived cytokines during early vs. established disease in the SAMP/Yit model of spontaneous ileitis. *Gastroenterology* 120:988 (abstract)
172. Mizoguchi A, Mizoguchi E, DeJong Y, Takedatsu H, Preffer FI, Terhorst C, Bhan AK. 2001. Role of CD5 expression on TCR  $\gamma\delta$  expression on TCR  $\gamma\delta$  T cell-differentiation and development of chronic intestinal inflammation. *Gastroenterology* 120:2629 (abstract)
173. Mizoguchi A, Mizoguchi E, Chiba C, Spiekermann GM, Tonegawa S, Nagler-Anderson C, Bhan AK. 1996. Cytokine imbalance and autoantibody production in T cell receptor-alpha mutant mice with inflammatory bowel disease. *J. Exp. Med.* 183:847–56
174. Mizoguchi A, Mizoguchi E, Bhan AK. 1999. The critical role of interleukin 4 but not interferon gamma in the pathogenesis of colitis in T-cell receptor alpha mutant mice. *Gastroenterology* 116:320–26
175. Takashi I, Kiyono H, Hamada S. 1997. CD4<sup>+</sup> T cell population mediates development of inflammatory bowel disease in T cell receptor alpha chain-deficient mice. *Gastroenterology* 112:1876–86
176. Iijima H, Takashi I, Kishi D, Kim JK, Kawano S, Hori M, Kiyono H. 1999. Alteration of interleukin 4 production results in the inhibition of T helper type 2 cell-dominated inflammatory bowel disease in T cell receptor alpha chain-deficient mice. *J. Exp. Med.* 190:607–15
177. Coffman RL, Reiner SL. 1999. Instruction, selection, or tampering with the odds? *Science* 284:1283–85
178. Mombaerts P, Clarke AR, Rudnicki MA, Iacomini J, Itiohara S, Lafaille JJ, Wang L, Ichikawa Y, Jaenisch R, Hooper ML. 1992. Mutations in T cell antigen receptor genes alpha and beta block thymocytes development at different stages. *Nature* 360:225–31
179. Andres PG, Beck PL, Mizoguchi E, Mizoguchi A, Bhan AK, Dawson T, Kuziel WA, Maeda N, MacDermott RP, Podolsky DK, Reinecker HC. 2000. Mice with a selective deletion of the CC chemokine receptors 5 or 2 are protected from dextran sodium sulfate-mediated colitis: lack of CC chemokine receptor 5 expression results in a NK1.1<sup>+</sup> lymphocyte-associated Th2-type immune response in the intestine. *J. Immunol.* 164:6303–12
180. Ranscht B. 1994. Cadherins and catenins: interactions and functions in embryonic development. *Curr. Opin. Cell Biol.* 6:740–46
181. Gottesman MM, Pastan I. 1993. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* 62:385–91
182. Leveille-Webster CR, Arias IM. 1995. The biology of the P-glycoprotein. *J. Membr. Biol.* 143:89–94
183. Satsangi J, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. 1996. Two stage genome-wide search inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7, and 12. *Nat. Genet.* 14:199–202
184. Yacyszyn B, Maksymowych W, Bowen-Yacyszyn MB. 1999. Differences in P-glycoprotein-170 expression

- and activity between Crohn's disease and ulcerative colitis. *Hum. Immunol.* 60:677–87
185. Neish AS, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL. 2000. Prokaryotic regulation of epithelial responses by inhibition of I $\kappa$ B $\alpha$ -ubiquitination. *Science* 289:1560–63
186. Gewirtz AT, Navas TA, Lyons S, Godowski JP, Madara JL. 2001. Cutting edge: bacterial flagellin activates basolaterally expressed tlr5 to induce epithelial proinflammatory gene expression. *J. Immunol.* 167:1882–85
187. Egger B, Bajaj-Elliott M, MacDonald TT, Inglin R, Eysselein VE, Buchler MW. 2000. Characterization of acute murine dextran sodium sulphate colitis: cytokine profile and dose dependency. *Digestion* 62:240–48
188. Dielemann LA, Palmen MJ, Akol H, Bloemena E, Pena AS, Meuwissen SG, Van Rees EP. 1998. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. *Clin. Exp. Immunol.* 114:385–91
189. Mashimo H, Wu DC, Podolsky DK, Fishman MC. 1996. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. *Science* 274:204
190. Panina-Bordignon P, Mazzeo D, Lucia PD, D'Ambrosio D, Lang R, Fabbri L, Self C, Sinigaglia F. 1997. Beta2-agonists prevent Th1 development by selective inhibition of interleukin 12. *J. Clin. Invest.* 100:1513–19
191. Malfait AM, Malik AS, Marinova-Mutafchieva L, Butler DM, Maini RN, Feldmann M. 1999. The beta2-adrenergic agonist salbutamol is a potent suppressor of established collagen-induced arthritis: mechanisms of action. *J. Immunol.* 162:6278–83
192. Van Assche G, Rutgeerts P. 2000. Anti-TNF agents in Crohn's disease. *Expert Opin. Invest. Drugs.* 9:103–11
193. Kishimoto T, Akira S, Taga T. 1992. Interleukin-6 and its receptor: a paradigm for cytokines. *Science* 258:593–97
194. Atreya R, Mudter J, Finotto S, Mullberg J, Jostock T, Wirtz S. et al. 2000. Blockade of interleukin 6 *trans* signaling suppresses T cell resistance against apoptosis in chronic intestinal inflammation: evidence in Crohn's disease and experimental colitis in vivo. *Nat. Med.* 6:583–88
195. Barbara G, Xing Z, Hogaboam CM, Gaudie J, Collins SM. 2000. Interleukin 10 gene transfer prevents experimental colitis in rats. *Gut* 46:344–49
196. Tomoyose M, Mitsuyama K, Ishida H, Toyonaga A, Tanikawa K. 1998. Role of interleukin-10 in a murine model of dextran sulfate sodium-induced colitis. *Scand. J. Gastroenterol.* 33:435–40
197. Steidler L, Hans W, Schotte L, Neirynek S, Obermeier F, Falk W, Fiers W, Remaut E. 2000. Treatment of murine colitis by *Lactococcus lactis* secreting interleukin-10. *Science* 289:1352–55
198. Nelson DR, Lauwers GY, Lau JY, Davis GL. 2000. Interleukin 10 treatment reduces fibrosis in patients with chronic hepatitis C: a pilot trial of interferon non-responders. *Gastroenterology* 118:655–60
199. Picarella D, Hurlbut P, Rottman J, Shi X, Butcher E, Ringler DJ. 1997. Monoclonal antibodies specific for beta 7 integrin and mucosal addressin cell adhesion molecule-1 (MadCAM-1) reduce inflammation in the colon of SCID mice reconstituted with CD45RB<sup>high</sup> CD4<sup>+</sup> T cells. *J. Immunol.* 158:2099–106
200. Kato S, Hokari R, Matsuzaki K, Iwai A, Kawaguchi A, Nagao S, Miyahara T, Itoh K, Ishii H, Miura S. 2000. Amelioration of murine experimental colitis by inhibition of mucosal addressin cell adhesion molecule-1. *J. Pharmacol. Exp. Ther.* 295:183–89
201. Ludviksson BR, Strober W, Nishikomori R, Hasan SK, Ehrhardt RO. 1999.

- Administration of mAb against  $\alpha_E\beta_7$  prevents and ameliorates immunization-induced colitis in IL-2<sup>-/-</sup> mice. *J. Immunol.* 162:4975–82
202. Herfarth H, Brand K, Rath K, Rogler HC, Scholmerich G, Falk W. 2000. Nuclear factor-kappa B activity and intestinal inflammation in dextran sulphate sodium (DSS)-induced colitis in mice is suppressed by gliotoxin. *Clin. Exp. Immunol.* 120:59–65
203. Hoffman MA, Drury S, Fu C, Qu W, Taguchi A, Lu Y, Avila C, Kambham N, Bierhaus A, Nawroth P, Neurath MF, Slattery T, Beach D, McClary J, Nagashima M, Morser J, Stern D, Schmidt AM. 1999. RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 97:889–901
204. Koh WP, Chan E, Scott K, McCaughan G, France M, Fazekas de St. Groth B. 1999. TCR-mediated involvement of CD4+ transgenic T cells in spontaneous inflammatory bowel disease in lymphopenic mice. *J. Immunol.* 162:7208–16
205. Hammer RE, Maika SD, Richardson JA, Tang JP, Taurog JD. 1990. Spontaneous inflammatory disease in transgenic rats expressing HLA-B27 and human  $\beta_2m$ : an animal model of HLA-B27-associated human disorders. *Cell* 63:1099–112
206. Bertrand V, Quere S, Guimbaud R, Sogni P, Chauvelot-Moachon L, Tulliez M, Lamarque D, Charreire J, Giroud JP, Coutier D, Chaussade S, Breban M. 1998. Effects of murine recombinant interleukin-10 on the inflammatory disease of rats transgenic for HLA-B27 and human beta 2-microglobulin. *Eur. Cytokine Netw.* 9:161–70
207. Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A. 2000. Failure to regulate TNF-induced NF- $\kappa$ B and cell death responses in A20-deficient mice. *Science* 289:2350–54
208. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D. 1995. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappaB. *Nature* 376:167–70
209. Erdman S, Fox JG, Dangler CA, Feldman D, Horwitz BH. 2001. Typhlocolitis in NF-kappaB-deficient mice. *J. Immunol.* 166:1443–47
210. Dohi T, Fujihashi K, Rennert PD, Iwatani K, Kiyono H, McGhee JR. 1999. Hapten-induced colitis is associated with colonic patch hypertrophy and T helper cell 2-type responses. *J. Exp. Med.* 189:1169–80
211. Dohi T, Fujihashi K, Kiyono H, Elson CO, McGhee JR. 2000. Mice deficient in Th1- and Th2-type cytokines develop distinct forms of hapten-induced colitis. *Gastroenterology* 119:724–33
212. Snapper SB, Rosen FS, Mizoguchi E, Cohen P, Khan W, Liu C-H, Hagemann TL, Kwan SP, Ferrini R, Davidson L, Bhan AK, Alt FW. 1998. Wiskott-Aldrich Syndrome protein-deficient mice reveal a role for WASP in T but not B cell activation. *Immunity* 9:81–91